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Memories along the longitudinal axis of a rodent hippocampus: acquisition and consolidation of variants of a spatial task

Livia de Hoz

Doctor of Philosophy
The University of Edinburgh
2000

Declaration

In accordance with postgraduate degree Regulation 3.8.7 of the University of Edinburgh, I declare that the work described in this document is my own, except where otherwise indicated, and that this thesis was composed by myself.

Livia de Hoz

To my parents,

que me han apoyado tanto
como me han empujado.

Caminante, no hay camino,
se hace camino al andar.
Al andar se hace el camino
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.

Antonio Machado

Abstract of Thesis

The mammalian hippocampus is a structure of the brain believed to be essential in learning and memory processes. A current controversy concerns whether it is involved in one unique memory process or is responsible for several related but dissociable functions. And irrespective of the function(s) there is controversy concerning its role in processes of memory consolidation. This thesis, divided in two parts, addresses these two issues.

Part I: Published studies have suggested that hippocampal involvement in spatial memory acquisition is restricted to the septal (or dorsal) part of the structure, a result that supports the idea that the septal and temporal (ventral) parts of the structure have different functions. In the first part of this thesis I explore further the possibility of functional dissociations along the septotemporal axis of the hippocampus, including the importance of commissural projections. Partial lesions are made to the septal or temporal parts of the rat hippocampus, on one side or both. The behavioural essay involves acquisition of a spatial task (variants of reference memory in the watermaze). Although the original septal versus temporal dissociation is replicated, variations of the task protocol (number of days and trials per day of training) reveal that the temporal hippocampus can also support spatial memory. Learning can be attained with as little as 30% of the hippocampus spared. The results support the idea that the hippocampus is responsible for a unique process to which the projections to the septal and temporal parts, as well as the commissural associations, contribute differently. This contribution could be dependent on the training protocol.

Part II: It is well established that damage to the hippocampus, across different species, can result in graded retrograde amnesia. This has be taken by some to imply a role in the consolidation, as well as the acquisition, of memories. The second part of the thesis describes a series of collaborative experiments in which the involvement of the hippocampus in acquisition, consolidation and retrieval of spatial memories is explored. Using an AMPA receptor antagonist the septal part of the hippocampus is temporarily inactivated during acquisition, retrieval or during the memory retention interval of a watermaze reference memory task. The results reveal the hippocampus is involved in all three memory processes when animals are tested 16 days after the end of acquisition. However it is believed that once a memory has been consolidated, its retrieval can occur independently of the hippocampus.

Animal experiments suggesting this involved lesions of the septal hippocampus only. In work reported in this thesis, lesions to the septal or the whole hippocampus are made at different times (1 day or 6 weeks) after acquisition. Using a novel memory testing protocol, the temporal 30% of the hippocampus was found to be sufficient in the retrieval of this memory in a time-independent manner. Animals given lesions to the whole of the structure could not be reminded of what they had learnt earlier at either interval. The results suggest that the whole hippocampus is necessary for the consolidation of memories acquired with an intact hippocampus and that at least part of the hippocampus is necessary for retrieval of memories. The results obtained in part two of the thesis could be dependent on the training and testing protocol as well as on the navigational aspects of the task.

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Chapter 1

General Introduction

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Chapter 1

General Introduction

1.1 Memory and the hippocampus

The faculty of memory has fascinated philosophers and researchers for centuries. Across history, it has acquired different shapes and lived in different places in the minds of its thinkers. It has travelled from the blood to the heart in the movement of 'pneumas' (Aristotle), from the heart to the brain in the form of 'vital spirits' (Galen), along the various ventricles (Nemesius, Saint Thomas Aquinas, Saint Agustine) towards the cerebellum (Vesalius) to reach, finally in 1664, the cortical gyri (Willis). Within the cortex, the mediotemporal lobe and, specifically the hippocampus, have long since been associated with learning and memory.

In this thesis, a series of experiments aimed to explore certain aspects of the role of the hippocampus in memory processes are presented and the results discussed. Two orthogonal but related issues are addressed and, therefore, the thesis has been divided in two parts. In Part 1, I explore the question of whether the hippocampus acts as a unity or is functionally differentiated along its longitudinal axis. Part 2 undertakes the issue of hippocampal dependent memory consolidation with special reference to spatial memory.

This chapter is intended as a general introduction to the particular questions addressed in the experimental chapters.

In 1957, Scoville and Milner published what was to be a landmark paper, cited since then as the key reference for the close association between memory and the mediotemporal lobe. This paper reported on a patient, H.M., who had undergone surgical removal of part of his temporal lobe, including the hippocampus, in order to

cure his severe epilepsy. As a consequence of this removal, he lost the capacity to remember events and facts of the previous years of his life (retrograde amnesia) and the capacity to acquire new memories (anterograde amnesia) (Corkin, 1965, 1984; Corkin et al., 1997; Milner 1965, 1968). Evidence from other patients (Penfield and Milner, 1958; and later Zola-Morgan et al., 1986) and from lesion studies in monkeys (Zola-Morgan and Squire, 1986; Parkinson et al., 1988; Zola-Morgan et al., 1989 a and b) suggested that damage to the hippocampus might be enough to generate memory deficits. Damage to surrounding structures, however, can increase the severity of the impairment (Mishkin, 1978; Murray and Mishkin, 1986; Zola-Morgan et al., 1993). In fact, the cortex surrounding the hippocampus (entorhinal, perirhinal and postrhinal cortices) is also known to be important in memory processes (Murray, 1992; Suzuki et al., 1993; Murray and Mishkin, 1998; Bussey et al., 1999).

The reason why memory research has focused so much on the hippocampus is partly that the amount of damage to the hippocampus is correlated with the memory impairment in amnesia (Milner, 1974). Also, in part, it is because the hippocampus has defined anatomical boundaries which have facilitated its study in isolation from the rest of the cortex. As a result, the detailed anatomy of the hippocampus has become known, beginning with the classical and comprehensive studies of Ramón y Cajal (1904). Other temporal structures, such as the rhinal cortices, are less clearly defined and their boundaries have only been recently identified in primates and rats. The position of the hippocampus in relation to other cortical structures, receiving inputs from a variety of areas, also make it an ideal candidate to deal with the requirements, such as associating information acquired via different sensory modalities, of the general type of memory processes attributed to the hippocampus.

What is the role of the hippocampus in memory? Although the exact nature of this role is unknown and the subject of controversy, it is agreed that the hippocampus is not involved in all forms of memory. It is known, for example, that it is not involved in procedural memory (memory for skills; Cohen and Squire, 1980; Squire et al., 1984) and that it is not required for short-term memory (Gaffan, 1974; Mishkin, 1978; Aggleton et al., 1986, 1988; Squire et al., 1988). It is generally accepted that

the hippocampus is involved in declarative memory, which is the term used for the explicit memory of general facts and every-day life events (Squire, 1989). However, it is not known whether its role relates to particular types of declarative memory or to a general type of processing required across the different types of declarative memory. But before reviewing what is known about its function it is important to situate the hippocampus, in anatomical terms, with respect to the rest of the temporal lobe.

1.2 The anatomy of the hippocampus

The hippocampus is an allocortical structure situated in the temporal lobe. It has the shape of a "C" whose two extremes run towards the rostral part of the brain, the dorsal running further than the ventral (Fig 1.1). Throughout the thesis I will refer to the longitudinal axis of the hippocampus on numerous occasions. This runs from 's' to 't' in Figure 1.1.c, where 's' and 't' are known as the septal and temporal poles respectively. For this reason the longitudinal axis of the hippocampus is also known as the septotemporal axis.

In terms of its internal organization, the hippocampus is constituted by a couple of four layered cellular formations: the dentate gyrus and the cornus ammonis (CA). The latter is in turn divided into CA3, CA2 and CA1, according to the size and organization of their cells. The hippocampus forms part of a larger ensemble of structures known as the hippocampal formation. This includes, in addition to the hippocampus, the subicular formation (pre and parasubiculum and subiculum) and the entorhinal cortex (Amaral and Witter, 1995). The reason why structures whose internal organization is so different (different cells types, 4 to 6 cellular layers) are grouped together is because, while the rest of the cortex is characterized by reciprocal connections between the different areas, the structures just mentioned are exceptional in that their different cellular groups are connected largely via unidirectional projections (Fig. 1.2).

The focus of this thesis is on the hippocampus rather than the surrounding structures. These are, however, closely related to the hippocampus both anatomically and

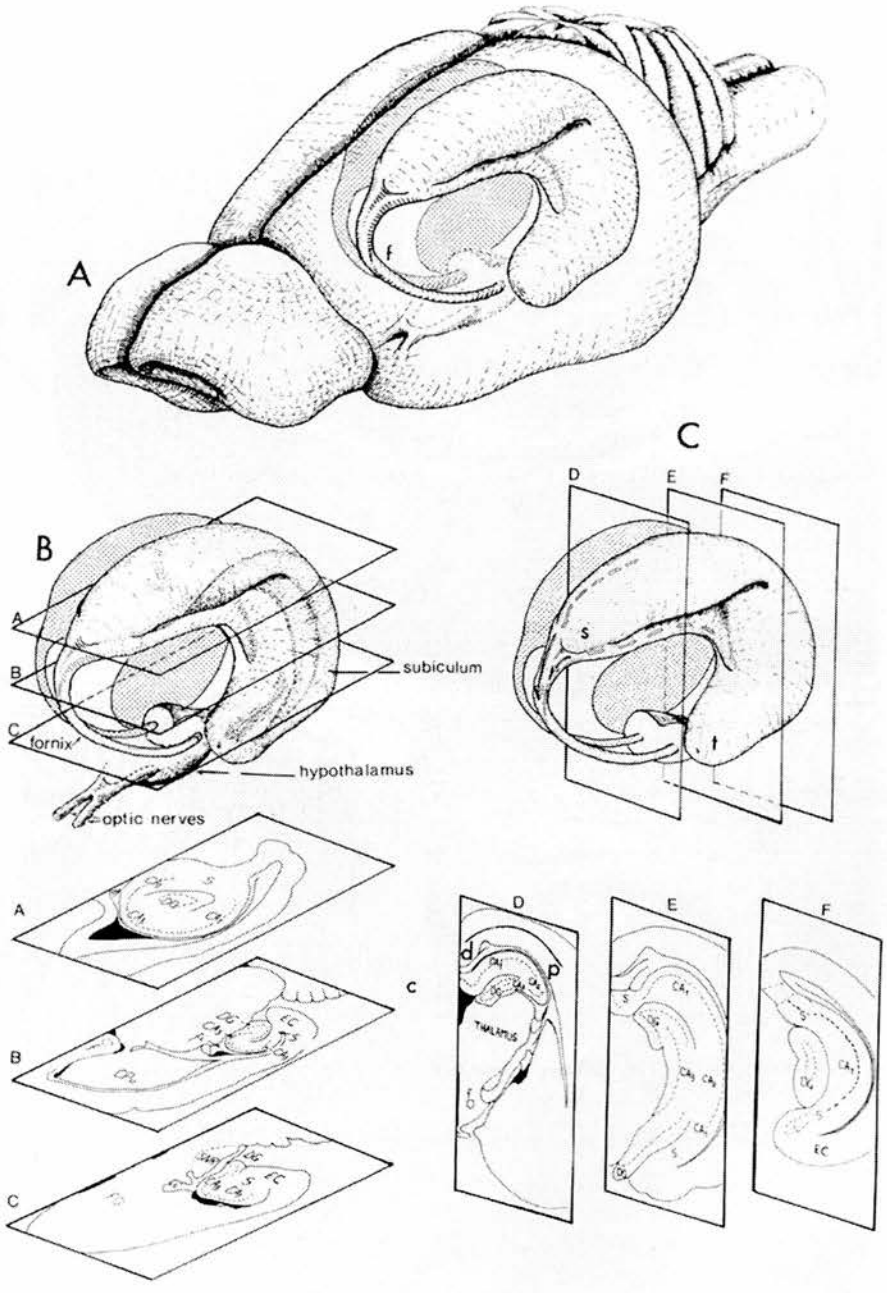

Figure 1.1: Schematic drawing illustrating the position of the hippocampus in the rat's brain (a) and the relative position of the different components as observed in horizontal (b) and coronal (c) sections. F: fornix; EC: entorhinal cortex; DG: dentate gyrus; S: subiculum; s: septal pole; t: temporal pole. From Amaral and Witter, 1995.

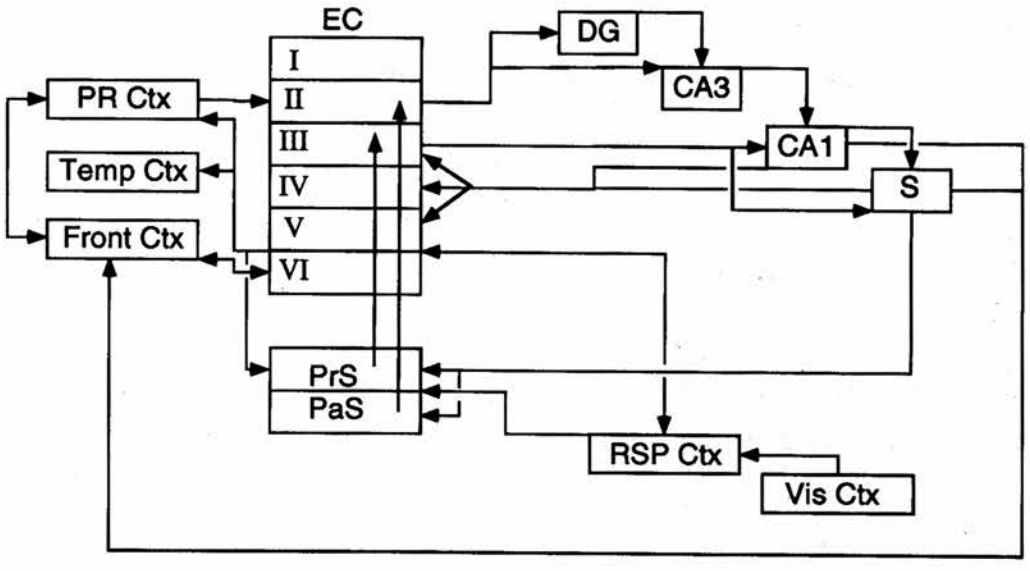

Figure 1.2: Schematic drawing illustrating the unidirectionality of connections between the different components (EC, DG, CA and S) of the hippocampal formation. EC: entorhinal cortex; DG: dentate gyrus; S: subiculum; PR ctx: perirhinal cortex; Temp Ctx: temporal cortex; Front Ctx: frontal cortex; PrS: presubiculum; PaS: parasubiculum; RSP Ctx: retrosplenial cortex; Vis Ctx: visual cortex. From Amaral and Witter, 1995.

functionally. In particular, the subiculum is generally understood to work together with the hippocampus as if it were part of the same structure. In fact, damage limited to the hippocampus in rodents generates a less severe deficit in spatial memory than that including the subiculum (Morris et al., 1990). As spatial memory is the subject of tests throughout this thesis, one could argue that it would be necessary to explore the role of both the hippocampus and the subiculum. However, damage limited to the hippocampus alone is enough to impair spatial memory (Morris et al., 1982; Jarrard, 1986) and, thus, it makes more sense to limit the scope of the research to one structure and avoid findings that cannot be clearly attributed to either the hippocampus or the subiculum.

The hippocampus is connected with both subcortical and cortical structures. Inputs are received, via the entorhinal cortex, from a wide variety of sensory associational cortices with the result that highly processed information across all modalities has access to the hippocampus. This is considered to be a key feature, in one way or another, in many theories of hippocampal function, specially by models of the hippocampus based on the structure's circuit (Treves and Rolls, 1992, 1994; O'Reilly

and McClelland, 1994; Levy, 1996; Rolls, 1996; McClelland and Goddard, 1996). The hippocampus, also mainly via the entorhinal cortex, projects back to these neocortical areas.

Subcortical inputs to the structure are, generally, understood to modulate hippocampal function. Although this is true of, for example, brains stem inputs, projections originating in the amygdala and hypothalamus do more than modulate its function. For example the amygdala is known to act in parallel with the hippocampus in memory processes underlying fear conditioning. As with the cortex, the hippocampus both receives inputs and sends outputs to these subcortical structures. These connections do not, however, necessarily occur via the entorhinal cortex and can be either direct or via other subcortical structures, like the septum.

The hippocampus is, therefore, in an ideal position to process a wide variety of associational cortical and subcortical information and to feed back to the structures this information originated from.

These and other aspects of the anatomy of the hippocampus are discussed in a later chapter but need to be taken into account during the brief introduction to hippocampal function presented now.

1.3 The function of the hippocampus

Although the function of the hippocampus has been the subject of extensive research for decades, the controversy is still enormous. A fair account of the research on hippocampal function would deserve a very extensive review. However, this thesis deals with two well defined aspects of the role of the hippocampus and, therefore, I have judged better to extend on detailed introductions to each of these aspects in future chapters and to limit this section to justifying the interest in the proposed questions.

Eichenbaum et al. (1994) propose that the hippocampus is involved in two orthogonal roles: the 'processing of a particular type of memory representation' and

the 'time-limited maintenance of memories'. Because this thesis is divided into two parts and each of them relates to one of the roles defined above, I will use the division proposed by Eichenbaum and colleagues to introduce both roles and the questions addressed in each part of the thesis. These sections are followed by a brief overview of the chapters that deal with them.

1.3.1 Hippocampal processing of a particular type of memory representation

It is generally accepted that the hippocampus is, among other things, required to deal with allocentric spatial information.

The spatial theory of hippocampal function was proposed by O'Keefe and Nadel (1978) in a book in which, based on behavioural and physiological data available at the time, they hypothesized that the role of the hippocampus was to generate a cognitive map of the environment. This map allowed the 'owner of the hippocampus' to navigate through the surroundings and make purposeful movements. Thus, according to the authors, the sole role of the hippocampus, with one exception, was to support spatial memory. The exception was verbal memory in humans, which they believed to be a type of memory with similar processing requirements to those of navigation and, thus, easily attributed to the hippocampus. This idea that the hippocampus first evolved to deal with spatial information and then became also capable of dealing with other types of information, is shared, in looser terms, by Squire (1993).

In the years following O'Keefe and Nadel's book, many studies have confirmed the role of the hippocampus in spatial memory. Rodents with lesions to the hippocampus are impaired in navigation and place-dependent maze solving (Morris et al., 1982). The same is true of monkeys (Gaffan, 1993; Parkinson et al., 1988; Angeli et al., 1993, Murray et al., 1998), although spatial problems suitable for primates have a somewhat different design to those used for rodents. It has been reported, however, that if a shaping protocol (training the animal in the different components of the task

step by step until acquired) is used, learning can occur even in the absence of a functional hippocampus (Whishaw and Jarrard, 1996).

Support for the spatial memory theory of hippocampal function is also found in the study of hippocampal cells. Recordings in rodents revealed that some hippocampal cells, named 'place cells', increase their firing frequency when the animal is in a particular position in space, which is in turn determined by a series of environmental variables (O'Keefe and Dostrovsky, 1971; Muller and Kubie, 1989; O'Keefe and Recce, 1993; Wilson and McNaughton, 1994; O'Keefe and Burgess, 1996; Jeffery et al., 1997). A similar type of response is observed in monkeys. In primates, however, the response is more closely related with the view from a particular location than with the monkey's position itself (Rolls and O'Mara, 1995; Rolls et al., 1998; Robertson et al., 1998). These cells are thus referred to as 'spatial view cells' (Rolls, 1996).

In addition to rodents and primate studies, evidence in support of the spatial memory theory can be found in studies of the avian hippocampus. The volume of hippocampus in migratory or food-storing species is larger in comparison with non-migratory or non-storing equivalents (Clayton and Krebs, 1995; Healy and Krebs, 1996). Moreover, changes in hippocampal volume seem to be experience-dependent and within the same specie the volume increases with the amount of spatial behaviour (Healy and Krebs, 1993; Healy et al., 1996).

The hippocampus is also involved in tasks that are considered to require spatial memory but cannot be easily interpreted in terms of cognitive maps. These are tasks in which the context is an important parameter. For example, Good and Honey (1991), trained rats in an operant task to lever-press in response to stimulus A in context C1 and to stimulus B in C2. Both sham and hippocampal lesioned rats were capable of acquiring those associations. However, when the two stimuli are presented together in context C1, only shams respond differentially to stimulus A. Rats with hippocampal lesions respond equally to both stimuli indicating that they have not established an association between each context and the stimulus that was presented in it during training. The context can in these circumstances be understood as the

spatial background in which associations take place. Nevertheless, rats with hippocampal lesions can learn to respond to stimulus A, but not B, in context C1 and to stimulus B, but not A, in context C2 (Good et al., 1998). This indicates that they can detect and use the context to respond flexibly to different stimuli. A similar finding is obtained in contextual fear conditioning. While rats with hippocampal lesions are unable to learn about contextual information when a stimulus is coupled with a shock in a particular context (Phillips and LeDoux, 1992), they can learn to detect and use the context when the context itself, and not the stimulus, determines the outcome of the task (Phillips and LeDoux, 1994).

That the spatial theory of hippocampal function does not cover the full processing capacities of the hippocampus became clear when, in other types of task, damage to the hippocampus revealed memory impairments that could not be interpreted as spatial memory deficits. For example, although rats with lesions to the hippocampus can learn certain relationships between pairs of stimuli (e.g., in a simultaneous presentation of stimuli B and Y, B is the rewarded one if the rat had been previously presented with stimulus A – represented as A, B>Y), they are impaired in flexible use across sets of relationships (Bunsey and Eichenbaum, 1996; Dusek and Eichenbaum, 1997) such as inferring transitivity (following the representation used above, if the rats have learned that A>B, B>C, C>D and D>E, when presented with B and D sham rats infer that B>D). These tests have no spatial component and, yet, the hippocampus is required for normal performance. Similarly, a functional hippocampus (plus subiculum) is required for retention of socially transmitted food preference (Winocur, 1990; Bunsey and Eichenbaum, 1995). This is a natural phenomenon by which, when a rat has to chose between two types of food, it will preferentially chose the food that was previously smelled on another rat's breath. Also, Davidson and Jarrard (1993) reported that lesions to the hippocampus impaired the use of interoceptive cues (such as hunger and satiation) to discriminate the relevance of certain stimuli. Rats were trained such that they received a shock in a chamber when they were hungry but not when they were satiated. Sham animals soon displayed differential freezing behaviour (one measure of fear) upon entering the chamber such that they froze more when hungry. Hippocampal lesioned rats, however, froze irrespectively of their previous food intake, showing that, although

they could detect the context, they were unable to associate this with differential hunger states in a flexible manner. None of the three tasks described above can be explained in spatial terms and yet they are hippocampal dependent.

Another example of a non-spatial memory process that can be affected by hippocampal lesions is the match and non-match type of tasks. In these tasks the subject is presented with two stimuli and is required to chose either the one that is identical to the stimulus presented previously (match) or the one that is different (non-match). I refer to this type of task for three reasons. First, it has been crucial in the development of hippocampal research. Second, it highlights the controversy surrounding hippocampal function. While certain laboratories report impairment following hippocampal lesions in humans, monkeys and rats (Zola-Morgan et al., 1986; Zola-Morgan et al., 1992; Wood et al., 1993; Alvarez et al., 1995; Rempel-Clower et al., 1996; Reed and Squire, 1997; Buffalo et al., 1998; Beason-Held et al., 1999; Manns and Squire, 1999; Zola et al., 2000), others find no deficit (Eichenbaum et al., 1994; Aggleton and Shaw, 1996; Vargha-Khadem et al., 1997; Murray and Mishkin, 1998; Aggleton and Brown, 1999). Third, whether the process is hippocampal dependent or not seems to depend on the manner the test is done. And, thus, when preoperative training is given the deficit can dissappear (Zola-Morgan and Squire, 1986; Babcock and Graham-Goodwin, 1997; Murray and Mishkin, 1998). This highlights the importance of the training protocol and the complexity of the memory processes underlying behaviour. For example, the matching or non-matching tasks have been generally interpreted as recognition memory tasks. There is evidence, however, suggesting that the hippocampus is not necessary for recognition memory (Bachevalier et al., 1985; Kowalska et al., 1991; Aigner et al., 1991; Meunier et al., 1997; Parker et al., 1997; Murray and Mishkin, 1998; Parker and Gaffan, 1998; Aggleton and Brown, 1999). Thus, either performance in the matching and non-matching tasks can, sometimes, rely on a process other than recognition (and this process is affected by hippocampal lesions), or the hippocampus is necessary for recognition memory in certain, non-established, circumstances. Also, as is discussed below, this deficit can be delay-dependent such that performance can be spared across short time-intervals.

Another example of a non-spatial task impaired by hippocampal lesions is an operant task trained with a differential reinforcement of low-rates (DRL) schedule (Rawlins et al., 1983). In this task the rat is required to lever-press to obtain food. This pressing, however, is only rewarded when, upon presentation of a stimulus, the rat waits a specified interval of a few seconds, before lever-pressing. This result led Rawlins (1985) to propose a theory of hippocampal function according to which the hippocampus was required for the processing of stimuli of all modalities when these are to be associated with other events that occur separately in time.

Finally, the hippocampus is believed to be the key structure underlying episodic memory, or memory for personal events (Tulving, 1993). Episodes or events are characterized, not by the elements that form them, but by the unique way in which they are combined. Remembering each of those unique combinations as separate is one of the roles attributed to the hippocampus. Unfortunately, this type of memory has, to date, only been characterized in humans (see Nadel and Moscovitch, 1997 for review of human cases of amnesia with episodic memory deficits).

The data presented above can be summarized and interpreted, in very general terms, as follows: the hippocampus

1. is involved in the rapid and flexible processing of stimuli of different modalities that are associated by spatial, temporal and/or logical (rule type) relationships.

2. is more likely to deal with one type of memory processing than with specific types of information as suggested by the fact that how the information is fed to the animal, the training protocol, can determine whether the hippocampus is required to solve a particular problem or not.

3. internal circuit is characterized by the processing capacities of pattern completion, pattern separation and comparisons. These features, although not discussed in this thesis, are likely to be essential for the type of processing proposed in point 1. The role of the hippocampus in episodic memory, memory for specific events in one's life, might be a consequence of its capacity to find

relationships and bind stimuli that are associated by spatial and temporal relationships but have to be distinguished from similar ones that happened in a different temporal frame.

4. its anatomy places it in the ideal position to receive all types of partially processed sensory and interoceptive information, supporting point 2.

That the hippocampus is involved in processing certain types of associations between stimuli is an idea shared by many theories of hippocampal function, known, for this reason, as 'binding' theories (Squire et al., 1984; Teyler and DiScenna, 1986; Moscovitch, 1995; Rudy and Sutherland, 1995; Wallestein et al., 1998). That the hippocampus is found to be active, in imaging studies, during mental processes that are not necessarily hippocampal dependent, supports the idea that this structure is involved in associating stimuli and events that happen across time and space. This is because in order to detect such associations the structure would not only need to record every event and process it together with contemporary stimuli but also with past and future ones.

The nature of its role in learning and memory processes is, however, still not understood. The controversy remains as to whether the hippocampus is involved in one general and abstract type of memory processing or whether it is involved in several related functions each of them dealing with a different type of information and/or a different type of memory processing.

A different approach to the understanding of the role of the hippocampus was taken by May-Britt Moser and colleagues (1993, 1995). They explored the possibility that different septotemporal levels of the rat hippocampus were differently involved in memory and found that while ibotenic acid lesions to the septal hippocampus impaired performance in a spatial reference memory task in the watermaze, lesions to the temporal hippocampus did not. Exploring whether spatial memory processing is limited to the septal part of the hippocampus is the subject of Part 1 of the thesis, the structure of which is discussed in section 1.5.

1.3.2 Time-dependent role in memory?

Human cases of amnesia can display temporary graded retrograde amnesia, such that while recent memories are lost, remote memories are spared (Ribot, 1881) . Once damage to the hippocampus was determined to be a characteristic feature of some forms of amnesia (Milner, 1974), it was suggested that the hippocampus played a temporary role in the maintenance of memories. Thus, it is believed that memories are dependent on the hippocampus only for a certain amount of time, after which they are probably dependent on the neocortex. The process by which memories become independent of the hippocampus with time is known as consolidation, a term coined in 1900 by Müller and Pilzecker, and later applied to the process described above (McDoughall, 1901; Burnham, 1903).

Little is known about the process of memory consolidation and the role played by the hippocampus. Retrograde amnesia can be the result of different degrees of damage to the hippocampus, can be caused by different forms of trauma, can cover from less than one to several years of the life of the patient and can affect different forms of declarative memory, semantic and episodic, differently. These are orthogonal issues that vary in, what seems to be, an independent manner between patients. The variability in human cases makes animal research particularly useful.

Animal studies using different hippocampus-dependent tasks support the idea of a time-dependent role of the hippocampus in memory (Zola-Morgan and Squire, 1990; Winocur, 1990; Kim and Fanselow, 1992; Anagnostaras et al., 1999). In spatial memory watermaze studies, however, lesions to the hippocampus result in a flat gradient of retrograde amnesia (Bolhuis et al., 1994 and Mumby et al., 1999a). Whether the latter is the result of the hippocampus being required for retrieval even after the memory has been consolidated, of a long consolidation process, or of the hippocampus being required for the navigational aspects of retrieval is not known. However it so happens that while watermaze studies have used complete hippocampal lesions, studies using other types of tasks have mainly involved lesions to the dorsal part of the structure. The flat gradient of retrograde amnesia found in

watermaze studies could thus be a result of the type of lesions used rather than of the task itself.

Part 2 of this thesis explores the role of the hippocampus in the consolidation of spatial memories and addresses some of the controversies outlined in the previous paragraph. A general overview of the chapters dedicated to this issue is given in section 1.6.

1.4 Lesion assessment

The approach used to address the questions posed throughout the thesis, both in Part 1 and in Part 2 with the exception of Chapter 9, was to explore the behaviour of rats with partial or complete lesions to the hippocampus in relevant versions of a spatial memory task in the watermaze.

In the case of complete lesions of the hippocampus it is important to establish that the damage is limited to the hippocampus in order to interpret the effect the lesion might have on the behaviour of the rat as a hippocampal dependent effect. Partial lesions require additional analysis, as it is important to establish that the damage does not affect the area of the structure that one wishes to spare.

For this reason, in Chapter 3, I present a general assessment of the lesion technique.

1.5 Part 1: a septotemporal gradient of function?

It has become apparent that the type of information received by the hippocampus is not homogeneously distributed along the septotemporal (longitudinal) axis of the structure. The septal (rostral, see Fig.1.1) pole of the structure receives a sensory input that is bigger in magnitude to that received by the temporal (caudal and ventral) pole. In addition, the temporal pole is differentially associated with the amygdala, a structure that deals with the emotional meaning of stimuli. For this reason the result by Moser et al. (1993, 1995), i.e. that lesions of the septal, but not the temporal,

hippocampus impair spatial memory, suggests that a functional differentiation might exist along the longitudinal axis of the hippocampus.

Anatomical and behavioural findings supporting the idea of a functional differentiation along the septotemporal axis are reviewed in Chapter 4. This leads to three experimental chapters.

In Chapter 5 the study by Moser et al. (1995) is replicated. The reason to replicate such an ostensibly convincing study is that surprising findings in Chapter 7 made it necessary. Thus, a selection of the groups used in Moser et al. (1995) were trained in a spatial reference memory task in the watermaze using a protocol identical to that used by Moser and colleagues. Consistent with previous findings, rats with septal hippocampus spared performed as well as shams during testing, while rats with temporal hippocampus spared were at chance.

Focusing on the group with septal hippocampus spared, one question is whether its ability to learn the task and display a spatial bias during testing is due the bilateral location of the tissue spared or whether an equivalent volume of hippocampus spared unilaterally could also support spatial learning. This question is addressed in Chapter 6.

In Chapter 7 the focus turns to the difference found between rats with septal and temporal hippocampus spared in spatial memory. Two approaches can be taken. One would be to assume that the results obtained by Moser et al. (1995) apply to all forms of spatial memory and, consequently, question what the role of the temporal hippocampus is. Another approach, the one adopted in this chapter, is to question whether the septotemporal difference applies to all spatial protocols and, thus, try to engage the temporal hippocampus in spatial memory. It is possible that, when the spatial demands are increased, the septal hippocampus is not enough to deal with the requirements of the task. In Chapter 7 rats with septal and temporal hippocampus spared are trained in two simultaneous reference memory tasks, each in a different watermaze situated in different rooms. The rationale behind this task is to maintain the protocol as similar as possible to that of Moser et al. (1995) but to increase the

difficulty, achieved by adding a level of spatial processing: distinguishing between two contexts.

1.6 Part 2: time-dependent role in spatial memory?

In Chapter 8 a review to hippocampal-dependent memory consolidation is presented and some questions on the subject introduced.

The first question addressed experimentally is whether the memory loss observed after hippocampal damage is due to a consolidation or a retrieval deficit. In Chapter 9, a collaborative study is presented in which, capitalizing on a new compound that permits the temporary inactivation of fast glutamatergic transmission in the hippocampus, the different memory processes (consolidation and retrieval) are explored independently. The role of the hippocampus in consolidation and retrieval, as well as acquisition, of spatial memory is, thus, explored using a reference memory task in the watermaze.

Following from the results obtained in chapter 9, further questions are addressed in Chapter 10. Watermaze studies to date find that lesions to the hippocampus result in a flat gradient of retrograde amnesia. It is possible, however, that after a lesion, the memory is not lost but merely inaccessible. This question is addressed by using a novel reminder protocol aimed to reactivate a possibly spared memory. Finally, it is often found in the human literature that data are interpreted as the result of an absence of hippocampal function when, in fact, part of the hippocampus is spared (Vargha-Kahdem, 1997; Reed and Squire, 1998; Teng and Squire, 1999). For this reason, in the same study, the outcome of complete hippocampal lesions is compared with that of partial hippocampal lesions.

Chapter 2

General Methods

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Chapter 2

General Methods

A description of all the methods used throughout the thesis is given in this chapter. Each individual experimental chapter will, nonetheless, contain a method section describing the variations applied to particular experiments. A brief scheme of different experimental designs is also outlined below. The methods are classified by type. Not every animal underwent all the protocols.

The generic structure of experiments investigating the differential role of septal and temporal hippocampus was as follows. Rats received partial, complete or sham ibotenic acid lesions of the hippocampus prior to the behavioural training. Training consisted of a reference memory task followed, in the unilateral versus bilateral study, by a delay matching to place task, both in the watermaze. The animals were then deeply anaesthetized and perfused and their brains removed and thoroughly analyzed to assess the extent of the lesion. A subset of the rats received retrograde tracer injections in the hippocampus a week prior to perfusion as a means of observing the pattern of residual connectivity of a partially lesioned hippocampus.

The temporary inactivation studies were organized as follows. Rats were trained in a reference memory task in the watermaze. An Atlantis platform was used in these studies. Some animals were implanted with LY326325 (LY) or aCSF micropumps and cannulae prior to the training for the inactivation of the hippocampus during acquisition and/or retrieval. Other rats were implanted with LY (or aCSF) micropumps after the behavioural training, at the start of the consolidation period. These rats were anaesthetized with halothane gas and decapitated, their brains sectioned, and the location of the cannula tip determined. The physical and temporal extent of the LY-dependent inactivation of the hippocampus was studied in a

different subset of animals by means of in vivo electrophysiological measurements and 2-deoxyglucose techniques.

The scheme for the lesion consolidation study was as follows. Rats were trained in a reference memory task in the watermaze using an Atlantis platform. They received complete, septal or sham hippocampal lesions either during the 2 days following the training or six weeks later. Testing occurred 2 weeks after surgery. Perfusion and histological analysis took place as described before.

2.1 Subjects and Housing

Male Lister Hooded rats were used in all experiments. They were supplied by the department breeding colony for the first year and by Charles River UK after the closure of the first. The weights ranged between 200 and 300g at the time of arrival. Each replication consisted of between 24 and 35 animals.

Animals were maintained in the laboratory animal room on a 12:12 hour light:dark cycle starting at 6 am. They were single housed throughout the experiment, with the exception of rats in the lesion consolidation study which were kept in pairs in group cages. They had *ad libitum* access to water and food (SDS rat diet RN1). The bedding consisted of dust free shavings and paper wool for nesting.

2.2 Surgery

2.2.1 Ibotenic acid lesion

The ibotenic acid lesion technique was first developed by Professor Len Jarrard (Jarrard, 1989) and is now widely used for lesioning a structure in a circumscribed manner while ostensibly sparing fibres of passage in doing so. These two properties are very valuable when doing partial lesions of the hippocampus, where control over the percentage of the structure to be lesioned and a sparing of the projections to and

from the intact portion of the hippocampus are both needed. Alternative methods are electrolytic and aspiration lesions. They are not recommended to lesion the hippocampus partially because the first cannot localize the damage, the latter damages the overlying cortex and neither spare fibres of passage.

Ibotenic acid is a neurotoxin derived from Mexican mushrooms (Sirakawa et al., 1966; Kishida et al., 1966 and 1967; Johnston et al., 1968; Konig-Bersin et al., 1970) that, when injected locally but not systemically (Kessler and Markowitsch, 1982), over-activates glutamatergic receptors and induces a massive release of calcium inside the cells, which die as a consequence of the increase in osmotic pressure (Olney, 1983). Ibotenic acid has also been reported to act upon the NMDA receptor and the trans-ACPD or metabotropic quisqualate receptor (Zinkand et al., 1992). These receptors have been implicated in neurotoxicity. There is also some evidence that it acts presynaptically as well as postsynaptically (Puil, 1981). It affects all excitatory neurons in the hippocampus, particularly CA1 neurons and leaves most interneurons intact (Wree and Erselius, 1991). The particular sensitivity of CA1 neurons to ibotenic acid (as well as to seizures and ischemia) could be due to the small synaptic clefts found in this area (McBain et al, 1990; Morris et al, 1991 and Vizi and Kiss, 1998). Grafted astrocytes do not survive an ibotenic acid environment indicating that it might not be just excitatory neurons that are affected (Fulop et al., 1997). Studies in the striatum have revealed that ibotenic acid does not affect the integrity of the blood-brain-barrier, unlike kainic acid (Nitsch and Hubauer, 1986); we may assume the same applies for ibotenic acid injected in the hippocampus. In principle, it leaves fibres of passage undamaged, but it has been observed that in the hippocampus (Erselius and Wree, 1991) a portion of the myelinated axons suffer from demyelination in the DG and CA regions as a consequence of an inflammatory response to the acid. Unmyelinated axons remain intact. Similar results had previously been found in the medial septum and lateral geniculate nucleus, although not in the caudate-putamen and fimbria fornix (Coffey et al., 1988). In the substantia nigra and pallidum, the neurodegeneration resulting from locally injected ibotenic acid is accompanied by the formation of calcium deposits (Herrmann et al., 1998). This effect has not been observed when ibotenic acid is injected in the striatum (Nitsch and Schaefer, 1990) or the medial septum (Saura et al., 1995). Ibotenic acid

coupled with hippocampal kindling enhances the stimulation of inositol phospholipid hydrolysis in slices (Iadorola et al., 1986). This reaction seems to be independent from the action of the amino acid over the excitatory receptor sites (Zinkand et al., 1992). Nakamura et al. (1992) have found deposition of amyloid beta-protein precursor in rat hippocampus lesioned by ibotenic acid. After a unilateral hippocampal ibotenic acid lesion, a reduction in glucose utilization is observed in the contralateral side of the hippocampus in addition to the side ipsilateral to the site of injection. This is explained in terms of an imbalance between dead excitatory cells and spared inhibitory cells in the ipsilateral side that affects the contralateral side (Zhou et al., 1995). Something similar might happen after partial lesions to the structure.

Ibotenic acid is a very useful tool when making partial lesions of the hippocampus but, as described above, it has negative side effects that need to be accounted for. Thus, it is very important to carefully assess the extent and efficiency of the lesion obtained and whether the spared tissue is healthy and maintains its normal pattern of connectivity. The methods used for this assessment are described in point 2.5 and the results are included in chapter 3. Although the retrograde tracing injections are part of the evaluation, they are included with the surgery methods rather than together with the dry techniques in point 2.5.

Surgical Procedure

The rat was anaesthetised with 1ml of avertin (a tribromoethanol-based anaesthetic) per 0.1 Kg rat weight injected peritoneally while under the effect of halothane gas. The top of the head was shaved and cleaned with alcohol. The animal was then placed in a Kopf stereotaxic frame with the mouth bar placed such that the bregma and lambda co-ordinates would rest in the horizontal plane. An incision was made longitudinally in a line mid-way between the ears. The skin was held at the sides with forceps exposing the top of the skull. Bregma co-ordinate was taken and compared with lambda. The points for the injections were calculated from bregma and marked over the skull. A dental drill was used to remove the bone overlying the injection points. This resulted in a 'c ' shaped hole over each hemisphere revealing the cortex overlying the hippocampus. Care was taken not to damage the dura during

the drilling and to regularly cool down the area with sterile saline to prevent the heat from the drill from damaging the cortex.

The dorso-ventral co-ordinate of the dura surface was then taken at a prearranged point and the cortex covered with sterile saline or with a piece of sterile absorbable gelatin sponge (Johnson, Southern Syringe) for the first and second hemisphere to be lesioned respectively. A 1µl SGE syringe was placed in the stereotaxic arm and filled with ibotenic acid (Sigma or Biosearch Technologies, 1mg/ml in sterile phosphate buffer saline, pH 7.4). Injections of 0.05, 0.08 or 0.1 µl ibotenic acid were made at points calculated from the Paxinos stereotaxic atlas (Paxinos and Watson, 1995) as in Jarrard (1989) with slight modifications to suit the borders of the partial lesions and our strain of Lister Hooded rats. A lesion affecting the septal 60% of the hippocampus would consist of 8 injections per hemisphere and a lesion affecting the temporal 60% of the hippocampus, of 6 injections. Both would amount to a total of 0.6 µl. A complete lesion would consist of 9 injections amounting to 0.91 µl per hemisphere. Some injections are 'double' in the sense that they occur at two dorso-ventral levels for the same lateral and rostro-caudal position. See Table 2.1 for coordinates.

Each injection involved lowering the needle through the dura and the cortex until the desired hippocampal depth was reached. The needle was left in place for 30 seconds to allow for the tissue, pushed down by the needle, to recover its original position. The injection was made at a rate of 0.15 µl/minute. After the injection the needle was slightly raised and left there for a further minute and a half to allow the ibotenic acid to spread. Finally the needle was raised very slowly (over aprox. a minute period) to stop the ibotenic acid from tracking back. Once removed the needle was cleaned with sterile saline to remove any trace of ibotenic acid. When both hippocampi were lesioned the hole over both hemispheres was covered with clean gelatin sponges before the skin was sutured back in place. The wound was covered in aureomycin powder. The rat was placed back in its home cage. The bedding was changed to a plain paper sheet until the rat's recovery was complete.

A sham lesion was done in exactly the same way except that no injections were made. The dura was pierced with a sharp needle in three points in each side, gelatin sponges were placed to substitute the removed skull and the skin was placed back together as previously described.

Complete lesion				**Septal 70% lesion**				**Temporal 70% lesion**			
AP	**L**	**V**	**µl**	**AP**	**L**	**V**	**µl AP**	**L**	**V**	**µl**	
-2.4	±1.0	-3.0	(0.05)	-2.4	±1.0	-3.0	(0.05)	-4.3	±4.0	-7.0	(0.10)
-3.0	±3.0	-2.7	(0.10)	-3.0	±3.0	-2.7	(0.10)	-4.8	±3.9	-7.0	(0.10)
	±1.4	-2.1	(0.05)		±1.4	-2.1	(0.05)		±5.4	-5.1	(0.10)
		-2.9	(0.05)			-2.9	(0.05)				
								-5.0	±3.0	-3.0	(0.05)
-4.0	±3.7	-2.7	(0.10)	-4.0	±3.7	-2.7	(0.10)				
	±2.6	-1.8	(0.05)		±2.6	-1.8	(0.05)	-5.7	±3.9	-3.6	(0.05)
		-2.8	(0.05)			-2.8	(0.05)			-7.0	(0.05)
									±5.0	-3.6	(0.05)
-4.3	±4.0	-7.0	(0.05)	-5.0	±5.2	-4.0	(0.05)			-4.9	(0.05)
					±3.9	-3.1	(0.05)				
								-6.15	±4.6	-4.6	(0.05)
-4.9	±3.9	-3.5	(0.05)	-5.5	±3.9	-3.6	(0.05)				
		-7.0	(0.10)								
-5.9	±5.1	-4.5	(0.08)								
		-5.3	(0.08)								
	±4.3	-3.9	(0.10)								

Table 2.1: Stereotaxic coordinates (in mm from Bregma; Paxinos and Watson, 1995) for complete and partial hippocampal lesions. AP: mm anterior-posterior from Bregma; L: mm lateral from Bregma; V: mm ventral from dura surface as measured at AP: -4.5 and L: ± 4.1; µl: of ibotenic acid to be injected at that site.
Other groups such as unilateral 30% or unilateral 60% (Chapter 6) were obtained by combination of a complete lesion on one side and a temporal 70% lesion or a temporal 40% lesion respectively on the other side.

2.2.2 Retrograde tracer injection

At the end of training, a subset of the rats received retrograde tracer injections in both the lesioned and the intact areas of the hippocampus as a mean of evaluating the integrity of the connections to the intact area. I used diamidino yellow and fast blue. Diamidino yellow is carried towards the cell body from the terminals where it is taken up and is then stored mainly in the nucleus. It gives a clear view of the labelled cell with no false labelling of the neighbouring cells. Fast Blue (Bentivoglio et al.,

1980) has similar characteristics with the difference that it labels the cytoplasm. The complementary storage (nucleus and cytoplasm) of these two tracers gives the opportunity of beautiful double labelling when combined together (Kuypers et al., 1980). Survival time, a critical value with many fluorescent dyes, is usefully long for diamidino yellow and fast blue (Keizer et al., 1983).

The surgical procedure is identical to that used for the ibotenic acid lesion but was performed after the end of training. Diamidino yellow (0.15 µl in one single injection) was injected with a 1µl SGE syringe into the septal hippocampus and fast blue (0.05 µl) into the temporal hippocampus. The rat was left to recover for 7 days before perfusion was carried out. Analysis of the retrograde tracer pattern was done under the fluorescent microscope by noting the areas of the brain that contained tracer, while blind to the type of lesion, and comparing these between the different lesion types.

2.2.3 Micropump implantation and chronic LY infusion

This procedure was carried out by Dr Gernot Riedel and Dr Steve Martin. The rats were anaesthetized and placed in the stereotaxic frame. The skull was exposed and a burr hole was made to allow the insertion of an L-shaped stainless steel cannula (26 gauge) bilaterally into the dorsal hippocampus (co-ordinates from bregma: AP = -4.5 mm; Lat. = ±3 mm; DV = -3 mm from dura). The cannula was attached to a 7 day osmotic micropump (Alzet 1007D) via flexible polyethylene tubing and secured in place with dental acrylic and three jeweller's screws. The length of the connecting tube was calculated to contain fluid for 20 hours (at a rate of 0.5µl per hour). The micropump was inserted into a subcutaneous pocket above the shoulder blades. Alzet 1007 micropumps, filled with LY (0.375 mM) or aCSF, infuse continuously at a rate of 0.5 µl / hr for 7 days.

2.2.4 Cannula implantation and acute LY infusion

The procedure was similar to the one described above and was also carried out by Dr Riedel and Dr Martin. Commercially available guide cannulae (24 gauge), dummy

cannulae, and injection needles were used (Plastics One). Guide cannulae were implanted bilaterally above the dorsal hippocampi (co-ordinates from bregma: AP = -4.5 mm; Lat. = ± 3.0 mm; DV = -3.0 mm from the dura). Four jeweller's screws were used to provide anchorage after cementing. Dummy cannulae consisted of a length of steel tubing attached to a plastic cap which could be screwed onto the guide cannula, resulting in an effective barrier against infection.

Prior to infusion, the dummy cannulae were removed from the guide cannulae and replaced by injection needles protruding approximately 0.2 mm below the base of the guide cannulae. These were connected, via plastic tubing, to microsyringes held in a syringe drive. A volume of 1 µl of LY (1.5 mM solution) or aCSF was infused into each hippocampus over a period of 5 min. The injection needles were left in place for approximately 1 min after the end of infusion.

In all the cases in which cannulae were used together with micropumps, the implantation was done simultaneously and the acute cannula (24 gauge) was soldered to the rostral length of the chronic one (26 gauge).

2.3 Behavioural tests- Watermaze

The watermaze (Morris, 1981 and 1984) was used as a behavioural test in all the experiments. The watermaze is an open-field navigation task. It is considered to be a spatial task and it is very sensitive to hippocampal lesions (Morris et al., 1982).

The point of the task is for the rat to find a hidden platform by swimming through the open-field. The platform constitutes the only escape the animal has from the water. As there are no intra maze cues, the rat has to learn the importance of the extra maze cues and how they relate to the platform's location. These cues then become the means for allocentric navigation.

The watermaze has been used for different purposes. It has proven very useful as a tool to test hippocampal function. When used in this way the performance of rats with permanent or temporary disruptions of the hippocampus is compared with that

of sham animals. It has been used by people investigating different aspects of hippocampal function: spatial memory (Morris et al., 1982), stress (Stillman et al., 1998; Hölscher, 1999), aging (Zyzak et al., 1995; Lindner, 1997) and pharmacological studies (f.e: NMDA-R involvement in learning and memory (Bannerman et al., 1995)). It has also been used to study navigation in itself and, in this case, it is usually done by training untreated animals in different variations of a task (Kavaliers and Galea, 1994; Moghaddam and Bures, 1996 and 1997). Other groups have used it to study the function of other higher order structures. These studies have revealed that the nucleus accumbens, for example, is not necessary to learn a reference memory task in the watermaze (Thifault et al., 1998) although lesions to the structure result in a slower learning rate compared to control animals (Annett et al., 1989). Involvement of the lateral pallidum (Jeljeli et al., 1999) and striatum in performance has also been highlighted and evidence that involvement is independent of that of the hippocampus (McDonald and White, 1994; Devan et al., 1996) has been presented. Prefrontal cortex lesions affect behavioural flexibility in the watermaze but not standard measures of spatial memory (de Bruin et al., 1994). Lesions to the mammillary nucleus impair performance in a working memory task, but not a reference memory task, in the watermaze (Santin et al., 1999). Lesions to the thalamus also result in impairments in the watermaze. Depending on the site of the lesion, these may extend to the visible platform task (Savage et al., 1997). They are, however, of different nature to those generated by fornix transections (Warburton and Aggleton, 1999). And, finally, despite its role in modulating hippocampal function (Packard and Teather, 1998), lesions to the amygdala do not affect watermaze performance (Sutherland and McDonald, 1990). The watermaze is commonly used for rats, but a number of laboratories have now developed it to be used with mice. This enables the study of transgenic mice behaviour (Silva et al., 1998).

2.3.1 The watermaze apparatus

The watermaze is a pool 2 meters in diameter and 60 cm in height (Fig. 2.1). It is made of fibreglass, the interior is coated with gelcoat and painted white. It sits 60cm above the floor level on a frame in the middle of a room with prominent extra maze

cues such as wall posters, cabinets and metal racks. The water reaches a level of approximately 40cm. Escape is onto a solid platform, 11cm in diameter, whose surface is only 1 or 2 cm below the water's surface. The water is made opaque by adding white latex (cempolatex liquid- Beaver Ltd). This makes the submerged platform invisible for a swimming rat. The rat's path is recorded by a recessed video camera overlooking the pool and the video signal relayed to a video recorder. This signal is fed to an image analyser (HVS VP112). The co-ordinates of the rat's path are sampled at 10 Hz by an Acorn computer running the 'Watermaze' software written by R. Spooner. The programme can obtain different measures, such as the time taken by the rat to find the platform (escape latency), the path lengths, the percentage time spent near the side-walls, the swim speed or the percentage time spent in a specified area of the pool.

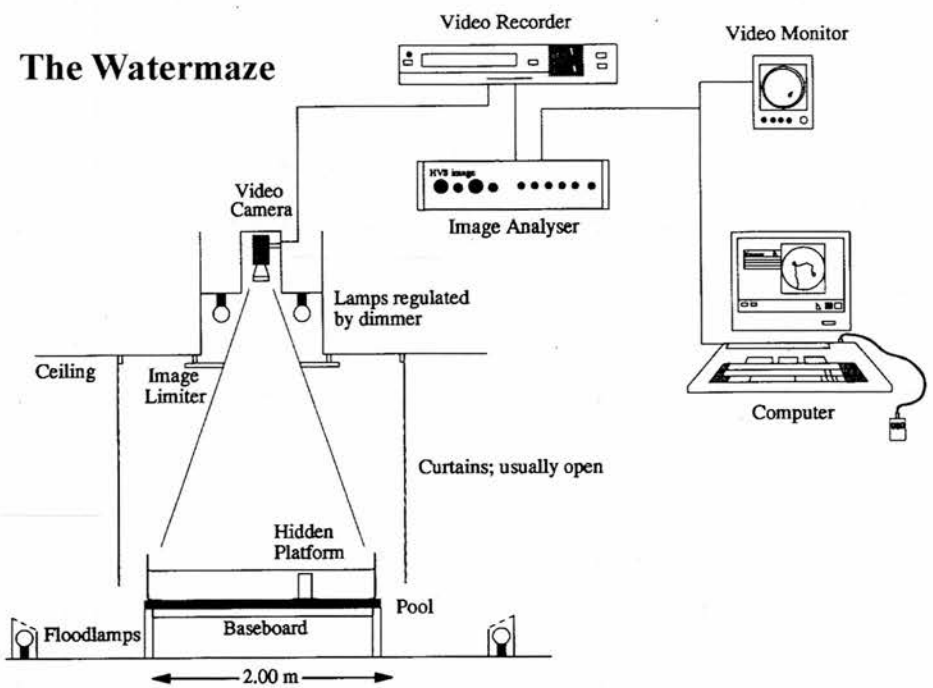

Figure 2.1: Schematic diagram of the watermaze and the equipment used to track the path of the animal. From Stewart and Morris, 1993.

2.3.2 The general protocol

The water is maintained at 25 ± 1°C (not too stressful but not too comfortable). In a normal trial, the rat is placed in the water facing the walls at any of four prearranged positions corresponding to the four cardinal points. The rat then swims in search of the platform. The rat is left on the platform for 30 seconds to allow it to look around and associate the platform position with extra maze cues. If the rat does not find the platform in 120 seconds it is led to it. This is sometimes the case on the first day of training but rare thereafter.

Two types of platforms were used, namely the 'standard' platform and the so-called 'Atlantis' platform. The 'standard' platform is a metal cylinder (11 cm in diameter and 27cm in height). It is fixed and stands just 1 or 2 cm below the water level. The rat 'bumps' into it when swimming in the area. The 'Atlantis' platform (Fig. 2.2; Spooner et al., 1994), on the other hand, is a foam cylinder (20 cm in diameter and 31cm in height) normally submerged close to the bottom of the pool. It is set to raise only when the rat dwells for a specific amount of time within a limited radius from the centre of the platform. After raising up it stands 1 or 2cm below the water surface. It is very efficient for reinforcing the rat's precision in its search.

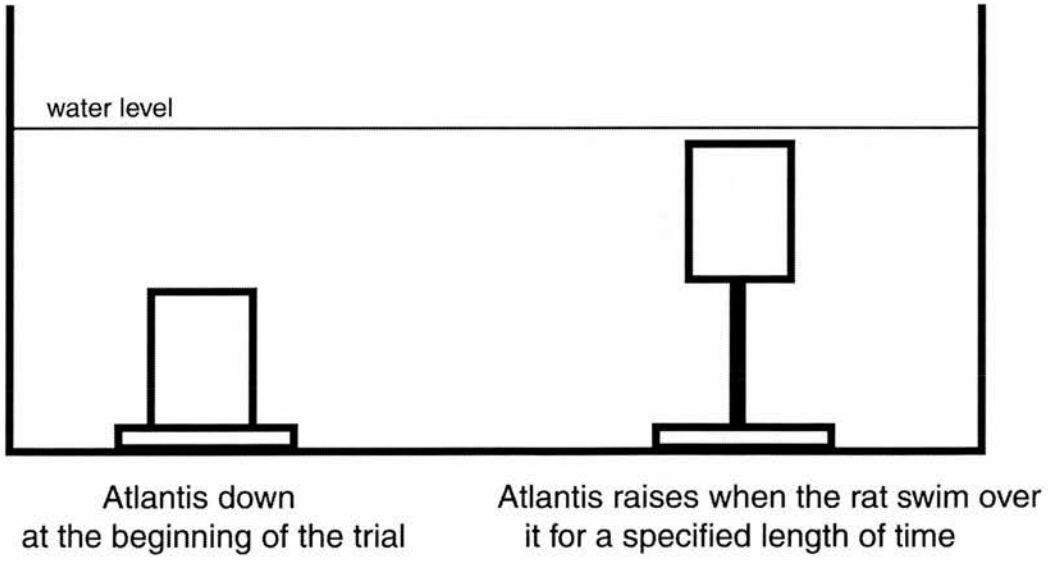

Figure 2.2: Schematic drawing illustrating the logic of the Atlantis platform.

A normal session generally consisted of four consecutive trials per rat. There could be one or two sessions in one single day. Alternatively, the session may be longer, consisting of 10 or 5 trials as was the case for the consolidation studies (LY and lesion respectively). At the end of each session, the rat is dried with a towel, taken back to the animal room and left under a heat lamp until dry.

2.3.3 Reference memory task

The idea of the reference memory task is that the platform's position is constant throughout the training, in the centre of the NE or SW quadrants of the pool (Fig. 2.3.). The rat has to learn that one and only one point in the pool is rewarded by escape onto a fixed platform. At the end of training, the rat undergoes what is called a transfer or probe test; this is a trial with no platform in which the rat is left to swim for 60 sec and the path is recorded. This test reveals whether the rat has a real knowledge of the platform's position. If so, it will spend a high proportion of the 60 sec in the quadrant previously occupied by the platform.

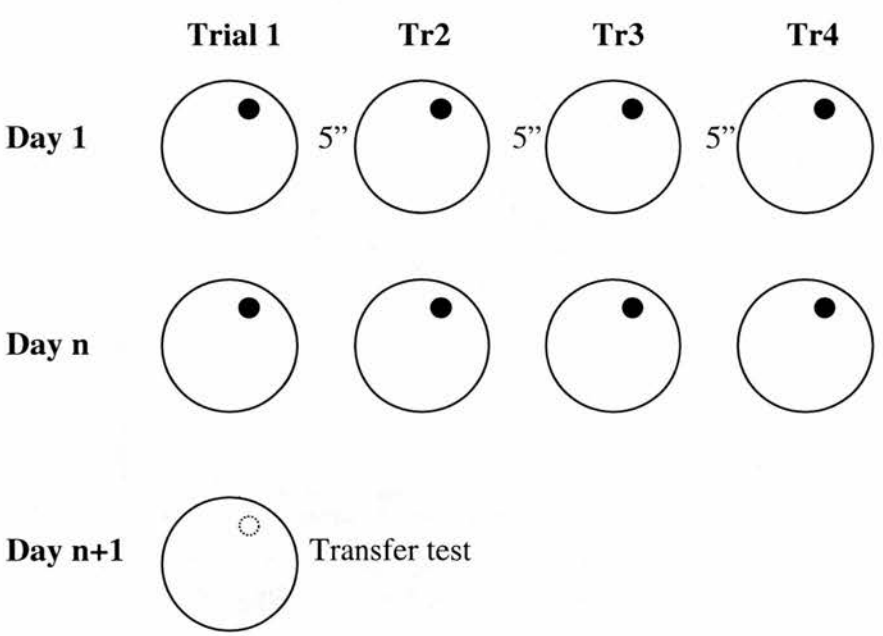

Figure 2.3.3: Schematic drawing illustrating the logic of the reference memory task and the final transfer test (when the platform is absent).

The platform position remains constant for each rat but different platform positions (NE or SW) are used for different rats to control for the possibility that certain areas of the pool might be more favoured by the rats than others.

The training in Part 1 of this thesis (functional differentiation along the septotemporal axis of the hippocampus) consisted of 6 or 8 days with 1 or 2 sessions per day. A transfer test was performed at the beginning of the 5^{th} day and on the day after the end of training (day 7 or 9). The training in Part 2 (memory consolidation studies) consisted of 4 days with 10 trials per day in 1 session (LY studies) or in 2 sessions (lesion studies).

Training in Part 2 of the thesis was preceded by a non-spatial pretraining given across 3 days (LY studies) or 1 day (lesion studies). The curtains were drawn around the pool to prevent the use of extra maze cues and a visible cue, in the form of a black and white cylinder, was hung over a variable (either of the 4 quadrants) platform position.

2.3.4 Delay-matching to place task

In this task (Steele and Morris, 1999) the rat receives four trials per day and the platform position is changed *quasi* randomly from day to day (Fig. 2.4). This means that on the first daily trial, the rat has no indication of where the platform is going to be. The performance on the second trial is a measure of the rat's memory for the platform's position in the first trial. The test is, therefore, one-trial memory. The third and fourth trials are given to prevent the possibility of extinction generated by a variable platform's position and to ensure that the performance of all animals is equivalent at the beginning of the following day. The latter is important when doing a within subject study of the differential effect of drug and aCSF infusion.

Some of the rats in Part 1 were trained in this way. The inter trial interval (ITI) between the first and second trials varied between 5 sec, 20 min or 2 hrs to study time-dependent lesion effects on memory. The rats were trained for 14 days, from which the first 5 were considered pretraining and were not analyzed in detail.

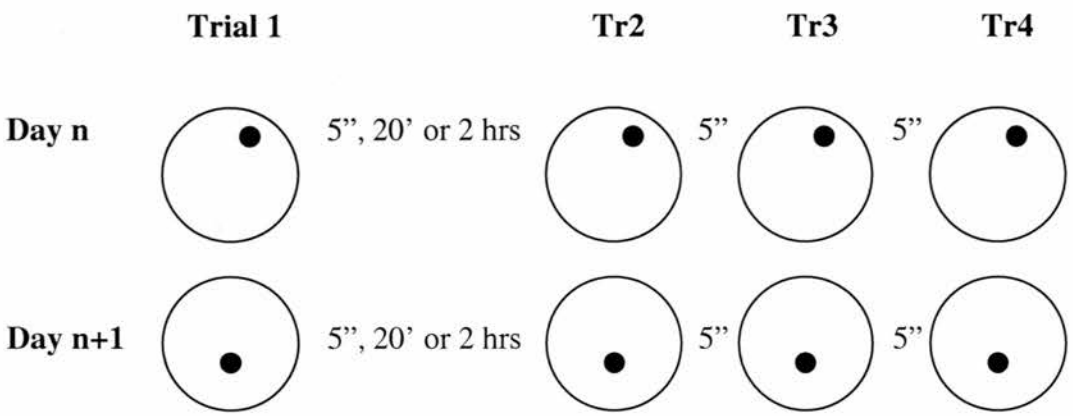

Figure 2.4: Schematic drawing illustrating the logic of the DMP task.

2.3.5 Visible platform task

After the reference memory task, a visually cued session was introduced in some of the studies. For this session, curtains were drawn around the pool to hide extra maze cues and a visible platform was used. The rat had to locate the visible platform and climb on to it. This task was used to make sure that all animals had unimpaired visual capacities and that any impairment found during training was due to the type of hippocampal lesion the animals had and not to any side effects of the surgical procedure.

2.3.6 Visual discrimination task

In this case, the watermaze is used for a non-spatial learning task. Curtains are drawn around the pool to prevent the use of extra maze cues. The rat has to discriminate between two cues of equal brightness only one of which had a platform underneath it. The cues are hung 10 cm above the water level and around 20 cm from the walls. They are 180° from each other around the perimeter of the pool and separated by a barrier running from the wall to the middle of the pool mid way between them. The rat has to go around the barrier back to the middle of the pool to go from cue to cue. The two discrimanda are either a black and white cylinder and a grey panel or a grey cylinder and a black and white panel. The cylinder was the rewarded cue in both cases.

2.4 Statistical analysis

All the watermaze studies were analyzed using analysis of variance (ANOVA). In case of heterogeneity of covariance of the within subject factor (the case of percentage time in each of the four quadrants of the pool), the Greenhouse-Geisser correction was used and is denoted in the text as 'corrected for sphericity' (Kinnear and Gray, 1999).

Newman-Keul's post-hoc multiple comparisons were generally used. However, when the study involved a high number of groups and the interest was on comparisons between particular groups, individual contrasts were made using the pooled error term, and analysis was limited to orthogonal sets.

A one-sample t test was used to compare a single group mean with chance level.

Categorical values are analyzed using Chi-square.

"SPSS" statistical package was used to analyze all data. Numerical values are stated as mean ± standard error throughout.

2.5 Lesion assessment

It is important to analyze the effect that ibotenic acid has in both the lesioned area and in areas it is not intended to lesion. Both a general evaluation of the technique and an individual analysis of each lesion and its behavioural effects can be made simultaneously.

2.5.1 Histology

At the end of each experiment the rats were either perfused intracardially with saline first and then with 10% formalin or decapitated. The brain was removed from the head and placed in 10% formalin until embedding and sectioning.

2.5.1.1 Embedding

For the first two replications of the septal versus temporal study and the micropump implanted brains, no embedding was done and the brains were cut directly after perfusion. Thereafter, the majority of the brains were embedded in fresh egg-yolk by Jane Knox. Only a subset was embedded by myself.

The embedding technique results in a much better and more homogeneous preservation of the shape of the brain. This is a feature that is most needed when measuring areas inside the brain. For this reason all the brains for the remaining lesion experiments were embedded.

Prior to embedding, the brains were blocked coronally, rostral and caudal to the hippocampus (aprox. 0.7 mm and –7.5 mm from Bregma respectively). Those brains that had received retrograde tracer injections were blocked at aproximately 1.7 mm and -10.8 mm from Bregma, in order to obtain sections from the septum to the locus coeruleus.

For embedding, the brain is placed in fresh egg yolk in a small ice-cube shaped open container. The cube is then placed over a tray with formalin in the incubator to 'breath' the vapours for 48 hours. This causes the yolk to harden around the brain. The plastic cube is then removed and the egg yolk block placed in formalin 10% for 2 days. The block is now ready to be cut.

2.5.1.2 Sectioning and staining

All the brains, whether embedded or not, were sectioned with a cryostat. Again Jane Knox carried out the vast majority of the sectioning. However, I also became competent in this technique and did some of the brains myself.

The brain was placed in the cryostat and 30 μm coronal sections were taken. Generally 1 in 5 slices was retained. If one section could not be taken it was recorded. The brains were preferably cut from posterior to anterior levels as this

leaves the right hand side of the brain on the right hand side of the section, when mounted directly onto the slide.

The sections were stained with cresyl violet and mounted with DPX. Two series of sections were recovered from the brains injected with retrograde tracer. One of these remained unstained and unmounted.

2.5.2 Volumetric Measurements

When doing partial lesions, it is important to be able to measure the exact volume of hippocampus spared. This ensures that animals can be properly grouped and that fair comparisons can be made between replications or, even, different studies. A more thorough discussion about the need to do this will be given in Chapter 3. The spared tissue in each lesioned brain and each sham lesioned brain was measured. Each section was placed under a makroscope or lens and the image imported into Canvas or the NIH Image system by means of a digital or a video camera. The spared hippocampal tissue was outlined and the area calculated (see criteria and Figures 3.1. a to c in Chapter 3). The total hippocampal spared tissue was calculated by summing the area of spared tissue for each section of that brain. The total area of each brain in a particular replication was compared with the average hippocampal tissue across sham lesioned rats in that replication and the percentage calculated.

Volumes could have been calculated by multiplying the spared hippocampal area by the distance between sections. However, this distance is identical for all animals and, therefore, the percentage tissue spared calculated from the total hippocampal volume spared would not differ from that calculated from the area spared. For this reason, it was decided to use the area measurement and not to do further step of calculating the volume.

2.5.3 Flat maps

The retrograde tracer results were studied using a Leica fluorescent microscope and a record of the structures containing fluorescent cells was made. The unstained

sections were used for this analysis. Some brains were selected for entorhinal flat maps. The flat maps were made following the protocol of Suzuki and Amaral (personal communication), in turn adapted from van Essen and Maunsell (1980) and Udin and Fawcett (1988). The idea is to convert the information obtained from all the coronal sections of a particular structure, in our case the entorhinal cortex (EC), into one map representing what the structure would look like if it had been taken out of the brain, unfolded along a cell layer and flattened. See Figure 2.5. for illustration of text below. A drawing of the outline of the brain was made with the Neurolucida system for each coronal section that contained the structure. The borders of the structure and the line of the layer along which the structure is going to be unfolded (layer II in our case) were marked on the same drawing. Cells filled with retrograde tracer were then plotted over this drawing. Neurolucida is very useful because it permits the plotting of entities visualized with different magnifications (from the outline of a whole section to the position of a particular cell) over the same drawing

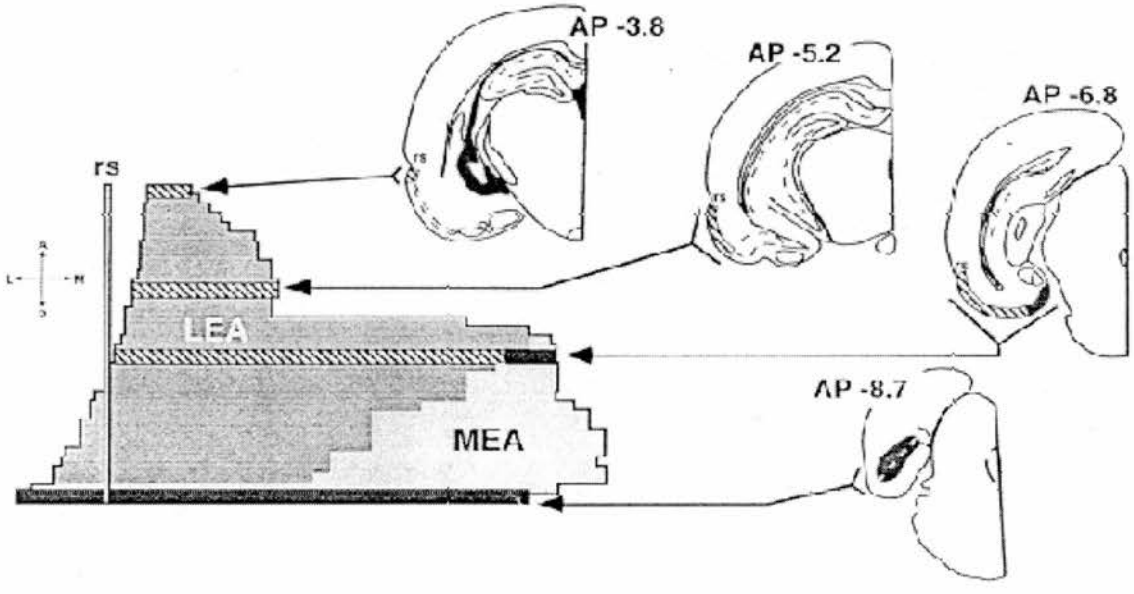

Figure 2.5: Drawing illustrating how coronal sections of the entorhinal cortex are unfolded to create a flat map of the structure. Ignore the division of entorhinal cortex into LEA and MEA. The rhinal sulcus (rs) is used as a reference point. A: anterior; P: posterior; L: lateral; M: medial; AP: anterioposterior position of the section from Bregma in mm. From Dolorfo and Amaral, 1998a.

while maintaining the correct spatial relationship between the different elements. The position of the rhinal sulcus with respect to the borders of the layer was also marked to be used as a reference point.

Once finished with the Neurolucida, the line that represented layer II in the EC was divided over the paper in segments whose length was the same as the distance between two sections (for the map to be proportional in length and width to the structure). The exact limits of the EC were determined with the aid of the parallel cresyl-violet stained series of sections. These were projected, using a Camera-lucida, over the corresponding section drawing and the limits marked over the drawing. The plotted EC cells were then allocated to the segment marked over the outline of layer II that was closer to them. When quantitative measures (topographical studies) are required, the number of cells allocated to each segment is noted. As the interest resided in qualitative assessment of the maintenance of the perforant path projection to different levels of the hippocampus, only the presence or absence of cells in the area surrounding each segment was noted.

The flat map was made over an Excel spreadsheet. Each row in the sheet corresponded to one section and each sheet-cell, to a segment. The borders of the layer in one particular section were marked on the excel sheet. If there were any plotted cells near a particular segment, this was denoted by a colour code on the sheet (no colour means no cells around that segment). The rhinal sulcus was used as a reference and was given a constant position along a column in the spread sheet such that the segments contiguous to the rhinal sulcus in different sections lie on top of each other on the sheet. Contiguous sections were given contiguous rows in the sheet such that the anterior section was above the posterior one. The figure obtained corresponds to a flat map of the entorhinal cortex with the retrograde tracer filled cells in their appropriate position in the map.

2.6 Temporary inactivation assessment.

Because of the temporary nature of the LY326325 effect on hippocampus, the evaluation of the inactivation has to be done in a separate group of animals from those that undergo behavioural testing. The technique may be assessed in two ways. To determine the time-course of the onset and the duration of the inactivation, *in vivo* acute and chronic electrophysiology was used. Field potentials in DG and CA1 were taken before and during the inactivation. Glucose uptake by the hippocampus during and after the inactivation was also studied to determine both, the physical extent of the inactivation and the amount of glucose utilization once the inactivation was over.

2.6.1 *In vivo* electrophysiology

The acute electrophysiology was carried out by Holly Bridge. Chronic electrophysiology was carried out by Gernot Riedel and Eva v. L. Roloff in the DG and Steve Martin and Beatrice Poeschel in the CA1 area.

The rats were anaesthetized with Urethane (acute experiments, 1.5g/Kg) or tribromoethanol (chronic experiments, 10ml/Kg), and, using standard stereotaxic techniques, implanted with teflon-coated stainless steel electrodes (75μm) to the appropriate depth of AP –7.5 and L –4.0mm (bipolar stimulation) and AP –3.5 and L –2.0mm (monopolar recording). Throughout surgery, all animals were placed on a heating blanket maintaining body temperature at 36.2 ± 0.2 °C. In acute experiments, a stable baseline (20min) was first obtained in response to electrical stimulation consisting of biphasic pulses with 100μs half-width delivered at 0.05Hz. Either aCSF or LY was infused as described in section 2.2.4 (Cannula implantation and acute infusion). Recordings continued for a further 6 hours.

In chronic experiments, an infusion cannula was inserted into the hippocampus at AP –4.5 and L –3.0mm from which a catheter was led to the micropump. Dental cement and stainless steel screws (one connected to the ground electrode) were secured to the skull. The micropump (ALZA 2002, capacity for 14 days long infusion) containing aCSF was placed as described in section 2.2.3 (Micropump implantation

and chronic infusion). After a recovery period of 7-10 days, the awake animals were placed in a recording chamber where they could move about freely while electrically connected via a swivel commutator to signal processing equipment. Daily recordings (3 days) included both I/O curves using stimulation varying from 100 to 1000μA (100μs half-width, 0.1Hz) and 10 minute baselines at fixed stimulus intensity (50% to 70% maximal response) with markers time-stamped to positions on the trace. Under anaesthesia, the micropump was then replaced with a another micropump (ALZA1007, 0.5μl/hr for 7 days) containing either aCSF or LY (0.375mM). Daily recordings continued in the same way for a further 12 days.

2.6.2 2-Deoxyglucose

The 2- deoxyglucose (2-DG) autoradiography was done by Amy Lam in Professor James McCulloch's laboratory in Glasgow University.

Rats previously implanted (4 or 11 days earlier) with intrahippocampal cannulae and 7-day LY micropumps were anaesthetized with halothane. Polyethylene cannulae were inserted into the right femoral vein and artery to allow injection of [^{14}C] 2-deoxyglucose and the sampling of blood respectively. The cannulae were then passed subcutaneously and externalised at the nape of the neck. Animals were allowed to recover for a minimum of 2 hours before intravenous pulse of 50 μCi [^{14}C] 2-DG (specific activity 55.0 mCi/mol, Amersham Life Science) in 0.7 ml saline was injected over 30 seconds. Timed arterial blood samples (approx. 100μl) were drawn at fixed time points over the following 45 minutes and the concentrations of [^{14}C] 2-DG and glucose in the blood samples were determined. Forty-five minutes after the isotope administration, the rats were sacrificed with euthetal, their brains removed and frozen. The brains were cut serially (3:10) into 20μm thick coronal sections and autoradiograms were generated by exposing the brain sections with medical X-ray film (Biomax^{TM} MR film, Eastman Kodak Company), together with a series of precalibrated [^{14}C]-methyl methacrylate standards. Local rates of glucose utilization were determined with quantitative densitometric analysis using a computer based densitometer (MCID, Imaging Research Inc.).

Chapter 3

Lesion Assessment

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Chapter 3

Lesion Assessment

3.1 Introduction

Standard hippocampal lesions are accepted when histological analysis reveals maximal damage to DG and CA regions and minimal damage to extra hippocampal structures. The assessment of partial hippocampal lesions, however, is more demanding. It is not enough to ensure that extra hippocampal structures are intact, it is also necessary to assess that the area of hippocampus that one wishes to spare is preserved and remains functional. Additionally, one might wish to make behavioural comparisons between the effects of partial lesions affecting different parts of the structure. In this case it is necessary to calculate the percentage of hippocampus spared to ensure that the comparisons are made between groups of hippocampal partial lesions that are similar in size.

In this chapter, I describe the criteria used to assess both complete and partial hippocampal lesions and the results obtained from the histological analysis of the lesions on which the behavioural results are based.

The chapter is structured as follows. First, I will describe the assessment of extra hippocampal structures in both complete and partial lesions, starting with overlaying cortex and following with entorhinal cortex and subiculum. Second, I will include the retrograde tracer analysis of the preservation of connectivity in the spared area of hippocampus. Third, I will describe how the percentage of hippocampus spared is calculated, I will compare my method with those used by other people and I will give reasons as to why I believe the method used here is the best. I will also describe how to group the animals according to the results of the calculations.

Figures 3.1.a, b and c are examples of partial or sham lesioned rats. The text below will refer to these figures to illustrate the different points.

3.2 Extra hippocampal structures

3.2.1 Overlying cortex

The mammalian hippocampus is located beneath the neocortex. In chapter 2 it was argued that ibotenic acid lesions are preferable to traditional aspiration lesions because, among other reasons, they preserve this overlying cortex. It is believed by some that the sole function of this part of the cortex is to protect the hippocampus (pub argument). More seriously, it is clear that aspiration of small parts of this area does not result in an impairment in watermaze performance (based on performance of, for example, the sham group in Moser et al., 1993), although larger lesions do (DiMattia and Kesner, 1988; Sutherland et al., 1988). However, the combination of even a small lesion to the cortex with a hippocampus-lesion might be more detrimental than a hippocampal lesion alone, especially in the case of partial lesions. For this reason hippocampal function is best assessed when no other structure is damaged and it is important to ensure that the needle tracts resulting from lowering the needle through the cortex into the hippocampus where the acid is delivered, do not generate cortical lesions.

When the brain is extracted from the rat's skull after perfusion, it is already obvious whether any such damage has occurred. As illustrated in Figure 3.2.a, a good lesion should leave only minimal traces on the surface of the brain. After the brain is sectioned and stained, the cortex and the portion of corpus callosum running between this and the hippocampus should appear intact with only small scars corresponding to the needle tracts. This is made clear in the lesion examples in Figure 3.1.a and c (arrow in Fig 3.1.a, section number 4).

During 10 months, mid way into my PhD, the lesions I (an others) did, generated an uncharacteristic amount of cortical damage. During the behavioural testing it became clear that there was something unusual about the rats, as their performance was

Temporal lesion- 3735

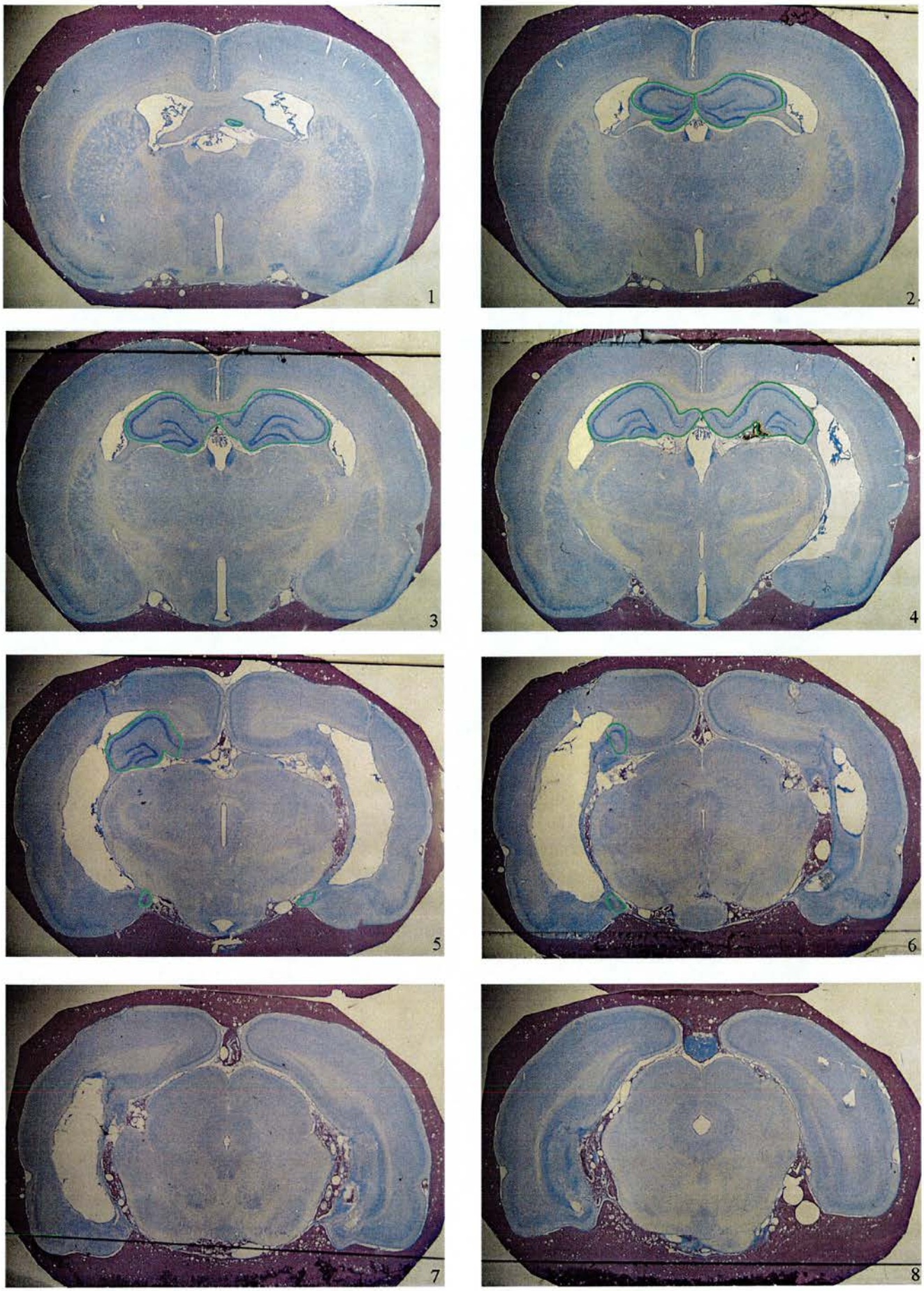


Figure 3.1.a: Evenly spaced coronal sections through the hippocampus where the septal level has been spared. Sections 1 to 8 run in the anterior posterior direction. The green outline contains the area of hippocampus considered whencalculating the amount of hippocampus spared.

Sham lesion- 3731

Figure 3.1.b: Evenly spaced coronal sections through the hippocampus of a sham lesioned rat.
Sections 1 to 8 run in the anterior posterior direction. The green outline contains the area of hippocampus considered when calculating the amount of hippocampus spared.

Septal lesion- 3761

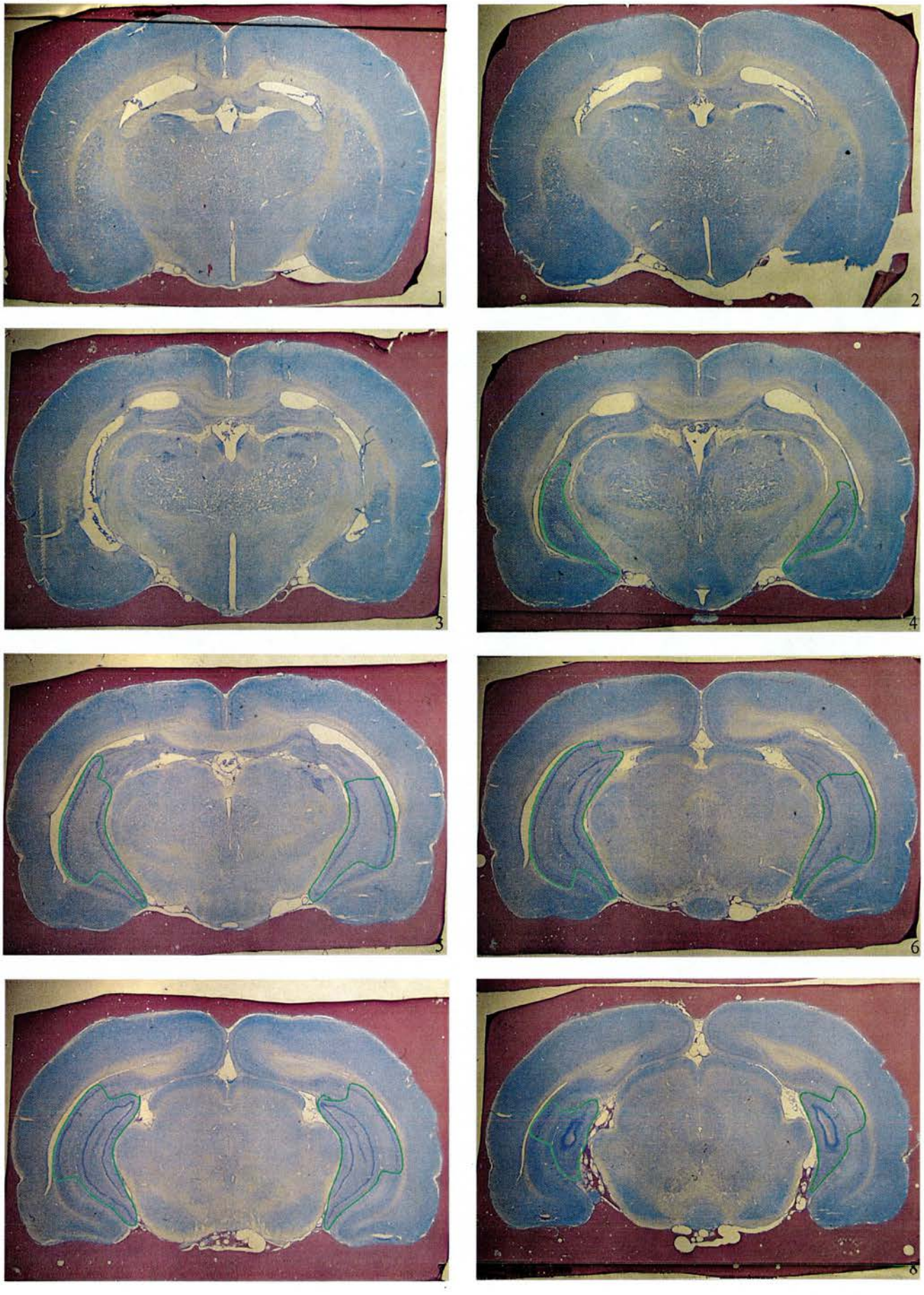
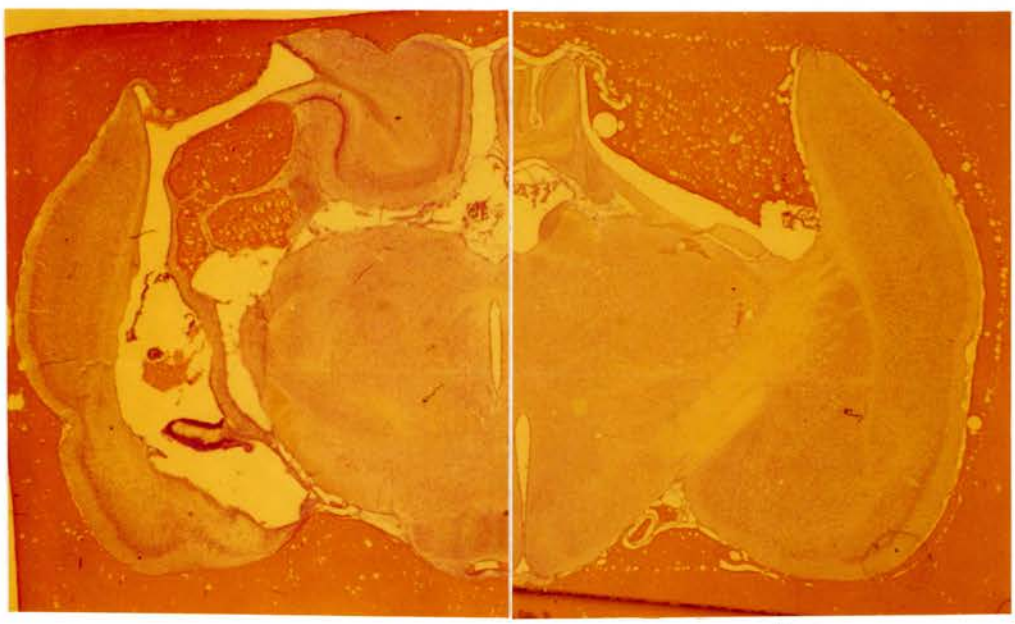


Figure 3.1.c: Evenly spaced coronal sections through the hippocampus where the temporal level has been spared. Sections 1 to 8 run in the anterior posterior direction. The green outline contains the area of hippocampus considered when calculating the amount of hippocampus spared.

a. b.



c.

Figure 3.2: Photographs of the brain of two hippocampus lesioned rats when removed from the skull after perfusion **(a and b)**. Examples of both, cortex unaffected by the lesion **(a)** and cortex damaged as a consequence of the lesion **(b)**. The latter was excluded from the experiment. Photograph of coronal sections across the hippocampus of two rats whose cortex was severely damaged as a consequence of the lesion **(c)**. Both were excluded from the analysis.

poorer than that of rats tested in previous replications (suggesting that partial lesions are more detrimental when combined with cortical damage). Consequent perfusion and histological analysis revealed that large areas of the overlaying cortex had been completely destroyed. This is illustrated in Figure 3.2.b and c. Three replications, one belonging to the study presented in Chapter 6 and two belonging to the study presented in Chapter 7, were completely discarded for this reason. The cause of this sudden failure, which was observed also in sham lesioned rats, is still unclear. It may have been due to faulty drilling equipment, some impurities in the ibotenic acid or other unknown factors. The positive outcome of this extremely frustrating episode was that I became more aware of the different aspects of the lesion protocol and its result and that my criteria became even tougher. As a consequence most of the lesions included in the behavioural experiments are from replications run after this episode.

3.2.2 Entorhinal cortex and subiculum

Hippocampal lesions were only accepted when the entorhinal cortex (EC) and the subiculum were spared.

Entorhinal cortex damage occured very seldom and only as a result of temporal or complete lesions. It was usually restricted to the medial portion of EC that lies ventral to the temporal hippocampus.

Subicular damage was also found occasionally as a result of temporal or complete lesions. This damage was usually unilateral and always restricted to the medial and ventral portion of the structure at the level where the subicular cell line appears to be a continuation of the ventral CA1 pyramidal cells. Damage to the dorsal part of the subiculum was not found in either septal or complete lesions. This is probably due to the spatial isolation (more medial) of this part of the subiculum from the rest of the hippocampus.

Hippocampal lesions were accepted if damage to the medioventral subiculum was mild and very restricted, as in the example in Figure 3.1.a (arrow, section number 7).

There are various reasons for this. As will become obvious in Chapters 5 to 7, rats with temporal lesions belong to a group whose mean performance is above chance in all the various watermaze protocols used. Thus, I am certain that small subicular damage does not cause behavioural impairments in the watermaze. To ensure that the behavioural variability found within this group was not correlated with subicular damage, all the rats were assessed and classified (while blind to their corresponding watermaze performance) according to whether they had mild subicular damage or not. There was no correlation (either positive or negative) between the presence of damage to the subiculum and performance of rats with temporal hippocampal lesions.

With these criteria it is ensured that the lesion is restricted to the hippocampus and that deviations in performance, as compared with sham animals, reflect hippocampal dysfunction.

3.3 Connectivity of the spared tissue

As discussed in the Methods chapter, it is important to ensure that the area of hippocampus spared in partially lesioned rats maintains its normal pattern of connectivity.

Retrograde tracer injections (see Methods, p. 26) were used to assess the status of the extrinsic connectivity of the residual hippocampus. The preservation of extrinsic connectivity in an animal with a septal lesion would indicate that the temporal hippocampus has not been de-afferented. In Moser et al. (1993), only rats with septal lesions were impaired, raising the concern that this might be the result of an effect of this type of lesion has over the connectivity of the temporal hippocampus, which courses, in part, through the septal hippocampus. Moser et al. (1995) examined the residual function of the temporal hippocampus electrophysiologically and using acetyl-cholinesterase histochemistry; but they did not do tract tracing.

The aim of the tracer injections is not to explore the detailed topography of hippocampal projections but, merely, to examine whether spared sections maintain

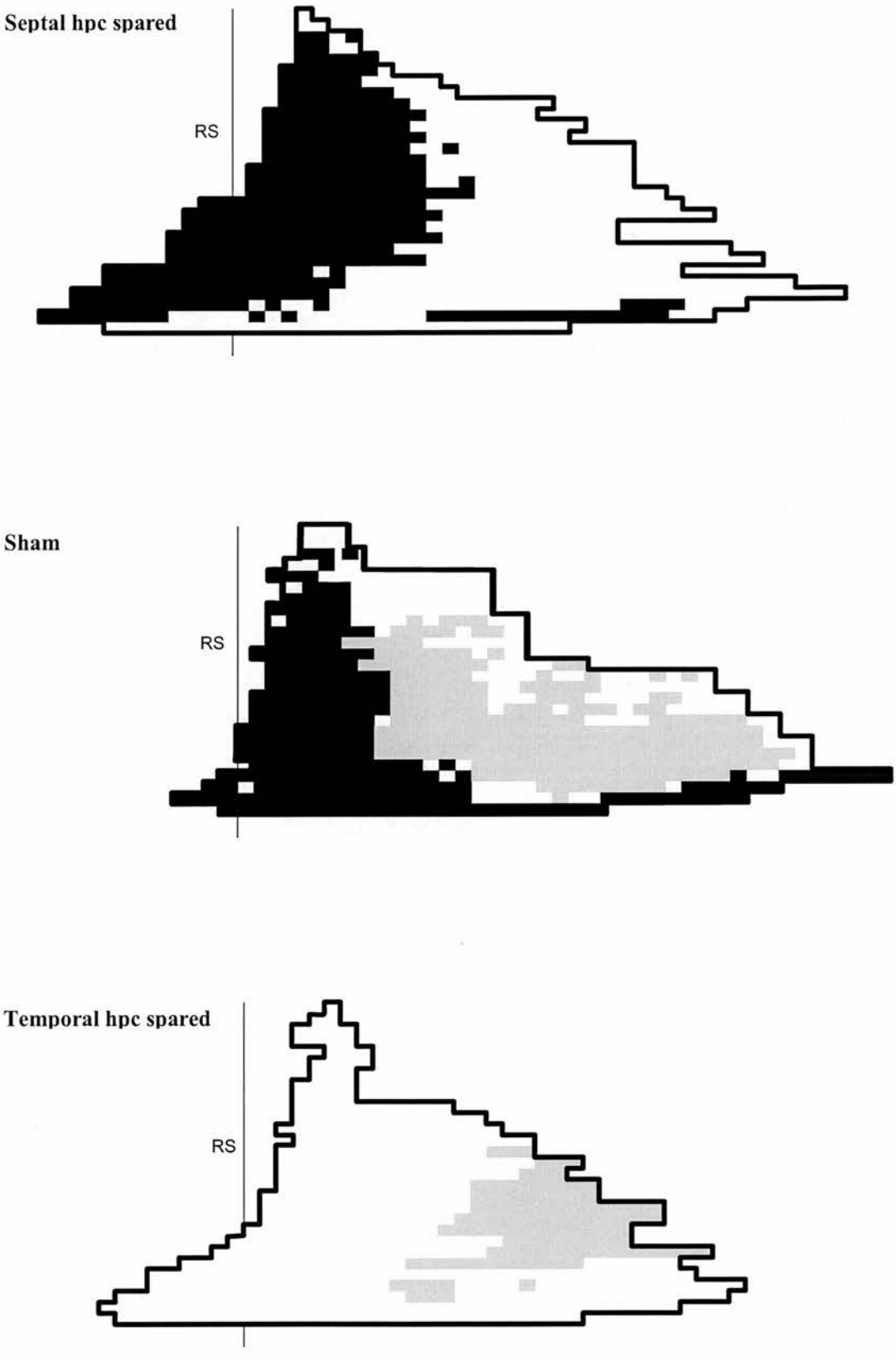


Figure 3.3: Flat maps of the entorhinal cortex of a septal hippocampus spared, a sham and a temporal hippocampus spared rat illustrating the pattern of labeling after hippocampal retrograde tracer injections. Dark and light gray represent labeling as a result of tracer injection in the septal and the temporal hippocampus respectively. RS: anterior posterior axis of the rhinal sulcus.

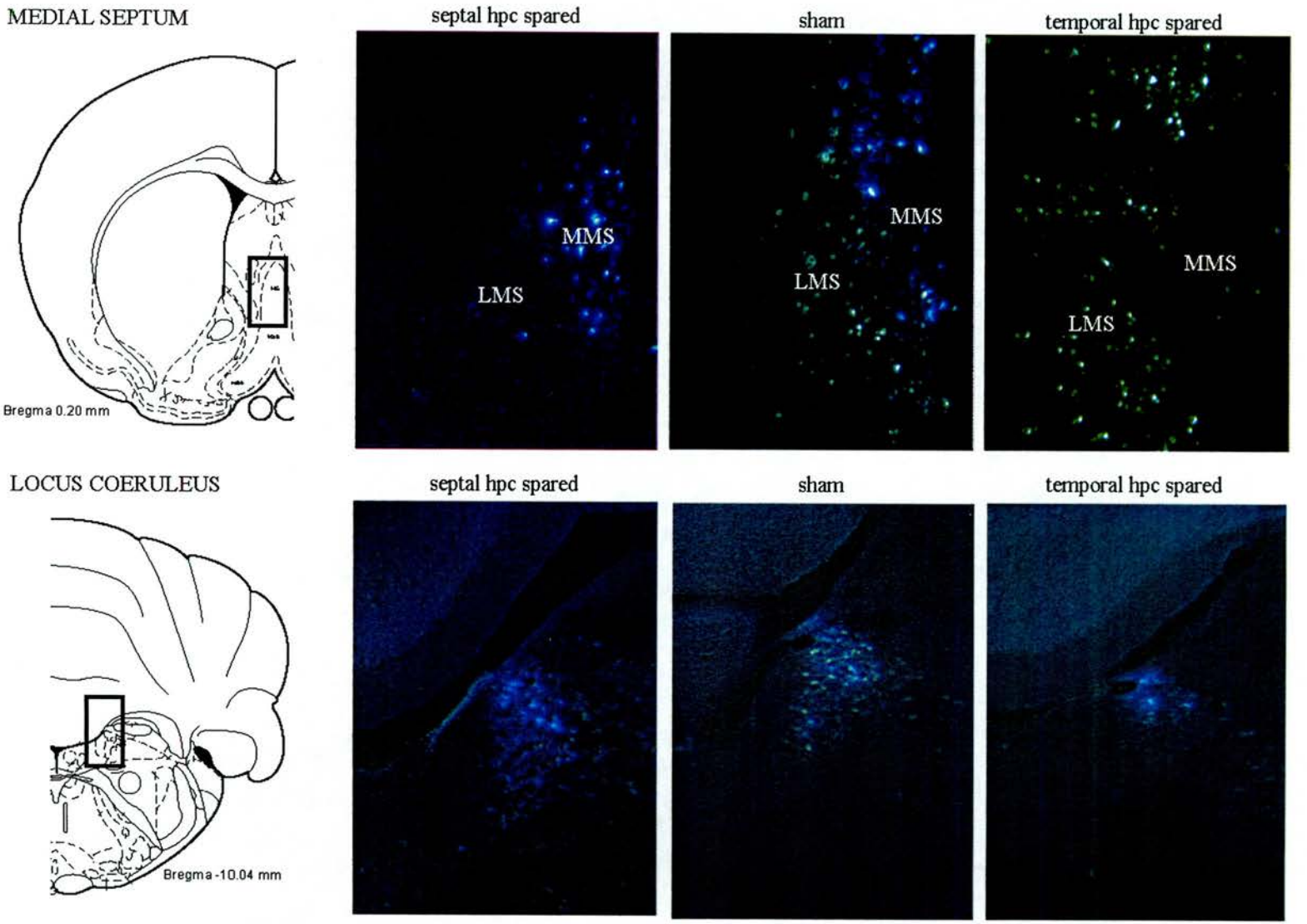


Figure 3.4: Photomicrographs of retrograde tracer labelling in the medial septum and locus coeruleus of partial and sham lesioned rats. Evidence for maintainance of inputs from these areas to the hippocampus and their topography. Blue labelling: fast blue; yellow: diamidino yellow. LMS: lateral medial septum; MMS: medial medial septum.

normal inputs. For this reason, description of each individual case has been omitted. Injection sites were consistent between rats and the most representative cases have been chosen for illustrations. In each brain injected with retrograde tracer labelling in the structures that project to the septal and/or temporal hippocampus (ie. entorhinal cortex, medial septum and diagonal band of Broca, locus coeruleus (LC) and raphé) was studied, as well as its topographical pattern, under a fluorescent microscope while blind to the type of lesion. The entorhinal cortex labelling was further studied by generating flat maps of the structure as described in the methods (p. 37).

In all cases observations matched what is known about the topography of the inputs into different parts of the hippocampus. In the case of the entorhinal cortex (EC) the pattern of labelling is represented over flat maps of the structure. In the three cases in Figure 3.3, the labelling accords to the topography of the entorhinal projection to the dentate gyrus (DG) described by Dolorfo and Amaral (1998a), i.e. that the lateral and caudal part of EC projects to the septal half of the DG, while the more medially and rostrally located cells in EC project to the temporal DG. Accordingly, when an injection was made in the temporal hippocampus, tracer was found in the more medial and rostral areas of EC. Also, an injection in the septal hippocampus resulted in labelling of the most lateral and caudal regions of EC. Moreover, lesions to either the septal or the temporal hippocampus did not affect the pattern of connectivity of the temporal or septal regions of the hippocampus, respectively.

Figure 3.4 shows photomicrographs of medial septum and LC. The septal and temporal hippocampus receive projections from the medial and lateral area of the medial septum, respectively (Amaral and Kurz, 1985). Accordingly, in rats with only temporal hippocampus spared, the lateral medial septum is labelled, while in rats with septal hippocampus spared, only the medial medial septum is labelled. The LC, on the other hand, projects to the whole septotemporal extent of the hippocampus (Haring and Davis, 1983). Accordingly, the LC appears labelled in both types of lesions.

These results confirm that the spared pole of hippocampus (septal or temporal) maintains its normal inputs and that these reach the hippocampus according to a characteristic topographical pattern.

As mentioned before, Moser et al. (1995), also driven by a concern over the functional state of the spared tissue, recorded from the spared hippocampus of animals with partial lesions to ensure that cells in this area displayed

normal responses. They found that their recordings were similar to those of sham rats. They also stained the tissue for acetyl-cholinesterase and found no difference between sham and partially lesioned rats. The anatomical finding presented here, thus, complement well the results described by Moser et al. (1995).

3.4 Percentage hippocampus spared after a partial lesion

Although this measurement is important in itself, it becomes essential when comparing rats with septal hippocampus spared with rats with temporal hippocampus spared. The three dimensional arrangement of the hippocampus in the brain results in the temporal hippocampus having a different orientation than its septal counterpart. Ideally, to ensure that measurements are not biased by this orientation, the hippocampus should be isolated from the rest of the brain, straightened and then sectioned. However, the necessity to assess the state of the surrounding areas and the connectivity of the spared hippocampus makes it imperative to preserve the rest of the brain and its spatial relationship with the hippocampus. For this reason calculations were made as described in the Methods chapter and illustrated in Figures 3.1.a, b and c (see legend). Areas of fibres such as the fimbria were not included in the outline of hippocampus spared. The reasoning behind this is that they would not be included in partially lesioned rats when everything else in that section would have been lesioned. Animals with the expected septal or temporal hippocampus intact, were discarded if they had more than 10% sparing elsewhere in the structure. Complete lesions were accepted when < 10% of the hippocampus was spared.

3.4.1 Why this method

This method is chosen over other possible methods because I believe it to be the most logical and reliable.

It is very similar to that used by Moser et al. (1993, 1995). However, their area measurements were based on projections of the cresyl violet stained sections over representation of the hippocampus in a Stereotaxic Atlas (Paxinos and Watson, 1995). This requires some subjective adjustments between the representation of the brain of a Wistar rat in the atlas and the observation of the partially lesioned brain of a Lister Hooded rat on the sections. Measuring the area directly over the sections is more reliable.

Other groups prefer to assess the percentage hippocampus lesioned, rather than that spared (Bannerman et al., 1999; Richmond et al., 1999). These calculations are based on comparisons between sections of the lesioned brain and corresponding sham sections or sections in a stereotaxic Atlas. The percentage obtained represents the percentage of the length of the longitudinal axis that has been lesioned. However, hippocampal cell death generates spatial distortions in the tissue that make the calculations difficult at times. In fact, Bannerman et al. (1999) required additional lesioned rats in order to establish the effect of the distortion and concluded that there was a positive correlation between time passed since the lesions was made and the amount of distortion in the spatial arrangement of the hippocampus. This method, therefore, also requires a certain amount of subjective judgement.

Hock and Bunsey (1998) divide the hippocampus in a septal and a temporal half and measure, in a fashion not dissimilar from that described in the Methods chapter, the percentage of septal or temporal hippocampus spared. However, there is no cellular or anatomical marker that differentiates between the septal and temporal halves of the hippocampus. This means that the method relies on the experimenter being able to decide what extent of the remaining tissue belongs to the temporal or septal halves and what percentage has been spared. In my view calculating the percentage spared as a fraction of a sham complete hippocampus is more reliable.

3.4.2 Methodological considerations

The method used here avoids the problems presented by the methods described above. There are, nonetheless, several aspects of the method that need to be considered carefully.

3.4.2.1 What is 100% hippocampus spared?

The percentage of hippocampus spared in each lesioned rat is calculated from the total area of sham hippocampus. The value used for sham hippocampus size is obtained by averaging the hippocampal area of all the shams. This is done within replications as variations in body weights and brain shrinkage as a result of embedding are smaller for the rats that belong to the same batch.

However, even within replications there is some individual variability in the size of the hippocampus. Thus, if the complete hippocampus of a partially lesioned rat was smaller or bigger than the average sham, the percentage spared obtained would have been bigger or smaller respectively. As within subject comparisons are impossible for obvious reasons, the best way around this problem would be to find a variable that is not affected by the lesion and correlates well with the size of the hippocampus such that the 'pre-lesioned' hippocampal size of partially lesioned rats could be inferred from this measurement.

The first candidate is the body weight at the time of perfusion. However, there is no correlation between this variable and the size of left (r: 0.18, df: 27, $p > 0.1$; Fig 3.4.a white triangles), the right (r: 0.33, df: 27, $p > 0.1$; Fig. 3.4.a black triangles) , or the combined left and right hippocampi (r: 0.24, df: 27, $p > 0.1$; data not shown) in shams and, therefore, the weight cannot be used to infer the size of the full hippocampus in a partial lesioned rat.

The next candidate is the size of the brain. However, no correlation was found between the width of the brain at a predetermined anteroposterior level and the size of the hippocampus in shams (data not shown). Nor was there a correlation between

Total hippocampal area against rat's weight

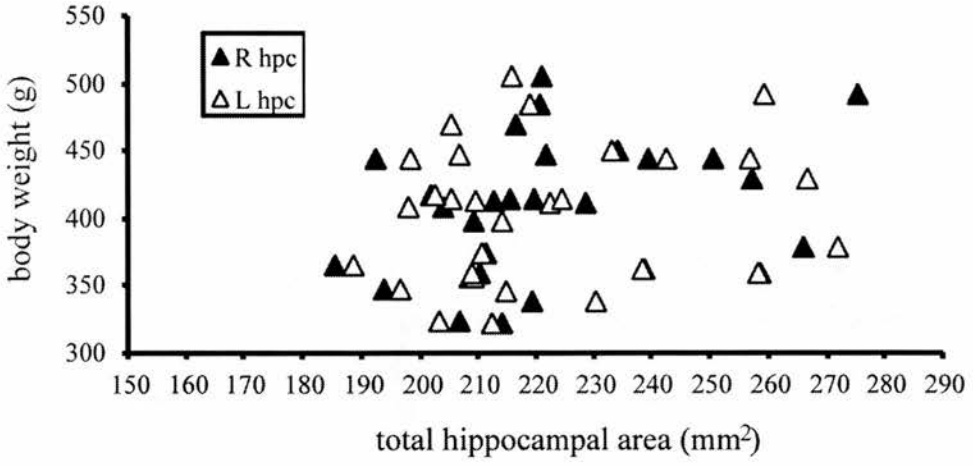


Figure 3.4.a: Sham lesioned rat's body weight at the time of perfusion plotted against the size (mm^2) of the right (R) or the left (L) hippocampus.

% hpc spared for different sham hippocampal sizes

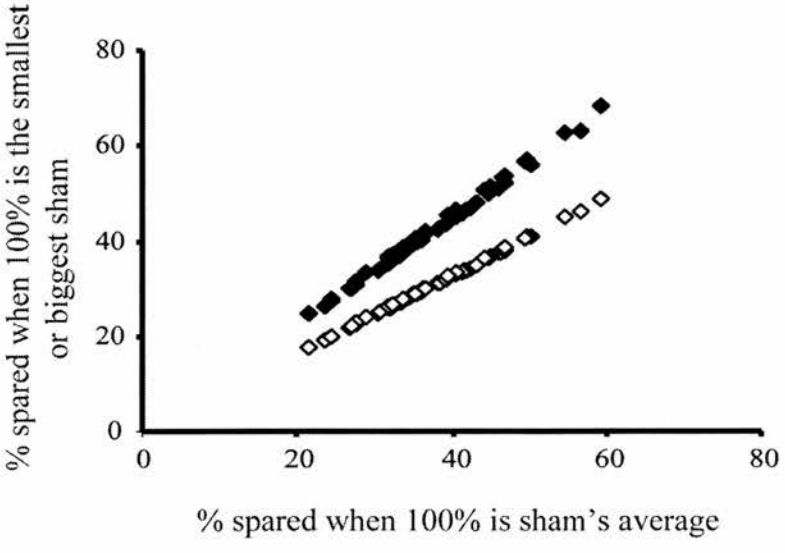


Figure 3.4.b: Graph illustrating how using the smallest (black diamonds) or the biggest (white) sham hippocampal size as 100% results in different % spared hippocampus in a subset of septal and temporal hippocampus spared rats.

the width of the brain at its widest point and the size of the sham hippocampus (data not shown).

What is the magnitude of this problem? Figure 3.4.b illustrates how using different sizes of sham hippocampus affects the final percentage spared value. It becomes obvious that the effect is bigger the smaller the lesion. If the rats with 20 to 40% hippocampus spared are grouped together, the risk of including a rat with more sparing only arises if that particular animal had a smaller than average hippocampus. Even then the extra sparing would be in the order of only 5%.

The conclusion is that the method used to determine the 100% value is the best in the circumstances and that the uncertainty generated by the limitations of this method constitutes a problem of small magnitude.

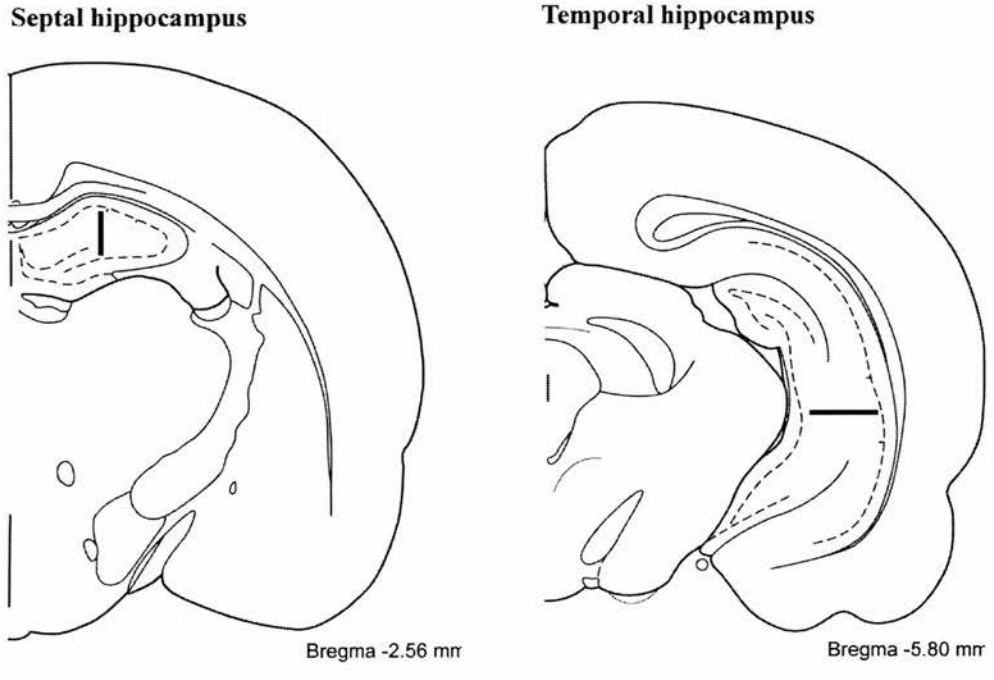


Figure 3.5: Distance between cell lines (dashed lines) is larger in the temporal hippocampus as indicated by the differential length of the black segment. Modified from Paxinos and Watson, 1998.

3.4.2.2 Are septal and temporal spared tissues equivalent?

Coronal sections disrupt the normal transverse outlook of the hippocampal longitudinal axis. It is possible that if the hippocampus were straightened and cut transversely along the longitudinal axis, the size of the total hippocampus obtained for sham lesioned rats would differ from that obtained here. In this case the size of the hippocampus spared would also differ. The question is whether these measurements would affect temporal hippocampal sections differently than septal hippocampal lesions. This is highly possible considering that coronal sections are orthogonal to the longitudinal axis of the hippocampus at its septal but not its temporal pole (in the rat).When looking at hippocampal sections it becomes clear that the amount of tissue between, for example, the DG and CA1 cell lines, is bigger in temporal sections (Figure 3.5). If this impression were true the length of cell line spared after partial lesions would be bigger in the septal than in the temporal hippocampus. This was confirmed when the length of the DG and CA cell lines was measured in a subset of the animals with different amounts of hippocampus spared septally or temporally. Figure 3.6.a and b, illustrate this and, although the difference is not big, it is important to keep it in mind. It is also revealed that, an increase in the amount of spared tissue results in a linear increase in the length of both DG and CA cell lines spared.

3.4.2.3 The grouping of rats according to percentage spared tissue

The percentage of hippocampus spared for each partially lesioned animal can be calculated in two ways. In Moser et al. (1995), this percentage is obtained by averaging the percentage tissue spared in each hemisphere. This decision was based on the need to compare our results with those of Moser et al. (1995). However, in Chapter 6 evidence is presented suggesting that rats with only unilateral tissue spared are capable of learning the watermaze task. This raises the possibility that the biggest percentage spared hippocampus out of the two hemispheres may be better reflection of the hippocampal 'capacity' of a particular animal. To be on the safe side, this is how the final individual percentage sparing is calculated in this thesis. Nonetheless, differences between hemispheres are small (average difference being 5.16 % for

Total CA length against total hippocampal area

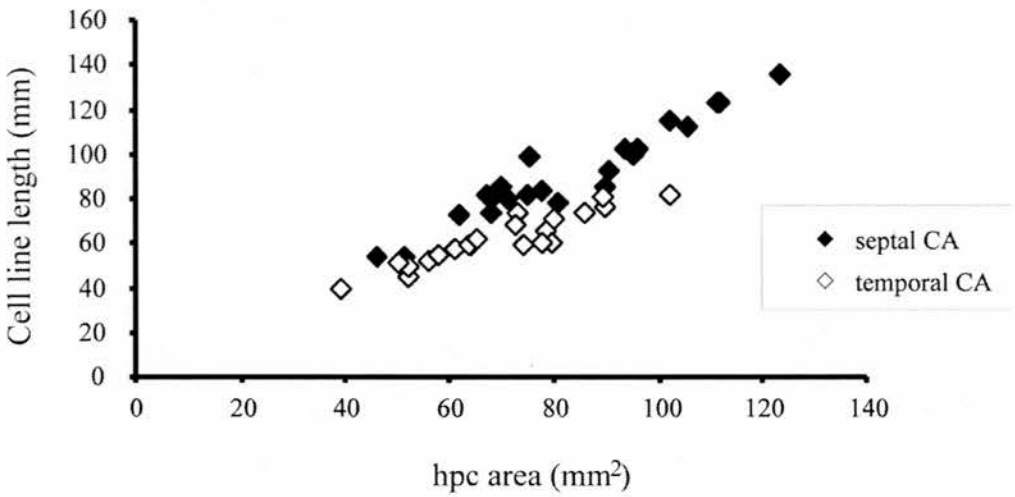


Figure 3.6.a: CA cell line length plotted against hippocampal size in a subset of rats with septal and temporal hippocampus spared. For a given area spared CA length tends to be bigger for animals with septal hippocampus spared than for those with temporal hippocampus spared.

Total DG length against total hippocampal area

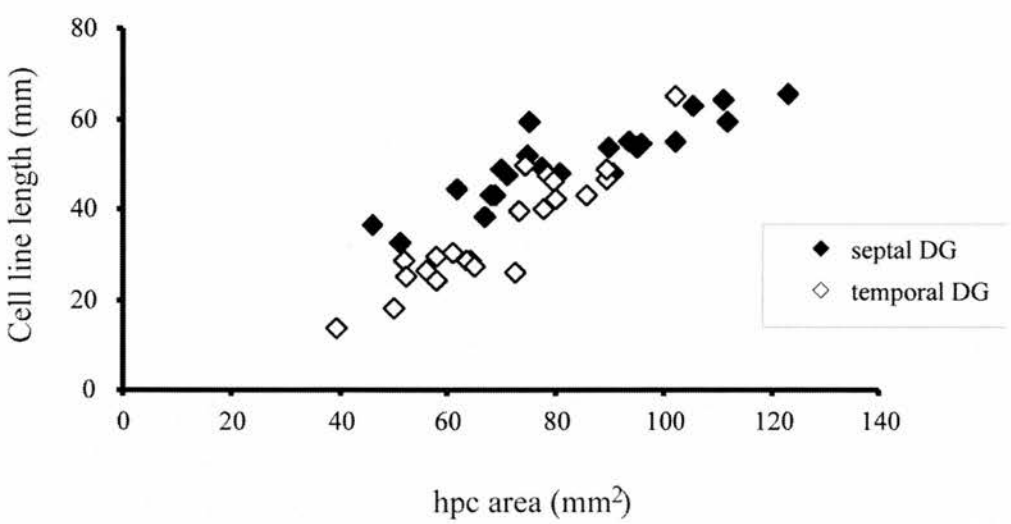


Figure 3.6.b: DG cell line length plotted against hippocampal size in a subset of rats with septal and temporal hippocampus spared. For a given area spared, DG length tends to be bigger for animals with septal hippocampus spared than for those with temporal hippocampus spared.

septal, and 6.62% for temporal hippocampus spared rats) and, therefore, the two methods result in similar values.

Different criteria can be used for grouping rats with amounts of hippocampus spared ranging from 20 to 80% starting from either the septal or the temporal pole of the structure. The first criterion is to separate septal and temporal hippocampus spared animals, because a comparison between these groups is the aim of the studies described in Part 1. Within these, Moser et al. (1995) put their animals into three groups: rats with 20 to 40%, 40 to 60% and 60 to 80% of the hippocampus spared. The primary focus of the data presented in this thesis resides in the septal and temporal 20 to 40% spared groups. The size of the resulting lesions was found to form clusters of septal 20 to 44% spared, temporal 20 to 41% spared and temporal 44 to 60% spared. It was decided to maintain these clusters rather than grouping the animals in strict 20 to 40% and 40 to 60% groups. In any case, the criterion of using the biggest hippocampus as the final percentage value means that the 20 to 44% group is similar to the 20 to 40% group defined by Moser et al. (1995), who used the average percentage value.

		sept hpc		sham		temp hpc		no hpc	
		% TQ	n	% TQ	n	% TQ	n	% TQ	n
Expt 1	20-40 unil	47 +/- 4	13	51 +/- 3	19	30 +/- 4	10		
	20-44 unil	46 +/- 3	16	51 +/- 3	19	30 +/- 4	10		
	20-40 bil	46 +/- 3	14	51 +/- 3	19	30 +/- 4	10		
Expt 2	20-40 unil	46 +/- 6	7	47 +/- 2	14	40 +/- 4	10	29 +/- 3	11
	20-44 unil	45 +/- 5	9	47 +/- 2	14	41 +/- 3	12	29 +/- 3	11
	20-40 bil	45 +/- 6	7	47 +/- 2	14	41 +/- 3	12	29 +/- 3	11
Expt 3	20-40 unil	39 +/- 5	6	56 +/- 2	17	40 +/- 4	11	28 +/- 2	9
	20-44 unil	39 +/- 4	9	56 +/- 2	17	41 +/- 5	13	28 +/- 2	9
	20-40 bil	39 +/- 4	9	54 +/- 2	21	38 +/- 4	13	28 +/- 2	9

Table 3.1: Effect of grouping method (unil: final value represents the biggest % spared of the two hemispheres; bil: final value is the average of both hemispheres) over a behavioural parameter (%TQ) and over the final 'n' values for three different experiments (Expt 1 to 3) in four different cases (sept hpc: septal hippocampus spared; temp hpc: temporal hippocampus spared; no hpc: no hippocampus spared).

Nevertheless comparisons were made using various different ways of dividing the animals into groups. As illustrated in Table 3.1, the different forms of grouping have no effect over a given behavioural measure.

3.5 Conclusion

In this chapter the assessment of lesions has been thoroughly discussed.

Lesions intended to be restricted to the hippocampus should be well defined as occurring primarily within it. Spared areas of hippocampus in partially lesioned rats are required to receive a normal set of inputs, with a characteristic topographical pattern.

The method used to measure the amount of hippocampus spared is, in my belief, the best possible. However, its problems are acknowledged and their magnitude assessed and proven to be of little consequence.

Part 1

The hippocampus longitudinal axis and its relevance for hippocampal function

Chapter 4

The hippocampal septotemporal axis

Table of Contents

Chapter 4

The hippocampal septotemporal axis

When one looks at the mammalian hippocampus, one can only marvel at the uniqueness of the organization of the different elements that form it. Whether looking at coronal or horizontal sections the pattern is equally recognizable and surprisingly similar across both types of cuttings. But, is the hippocampus functionally heterogeneous despite this homogeneous appearance? Furthermore, is the anatomy across the structure as similar as it seems?

In 1934 Lorente de No (1934, p.174) proposed that "the Ammon's horn, in spite of the uniformity in structure in its entire length, is an aggregate of similar organs receiving different afferents". However, in 1964 "the prevailing view of the hippocampus is still as a uniform structure throughout its length" (Elul, 1964a).

Since then, numerous anatomical and a few physiological and behavioural studies have confirmed that the hippocampus is not a homogeneous structure and that the differences appear mainly along the septotemporal (or longitudinal) axis. Chapter 4 is a review of the literature addressing the issue of a possible anatomical and/or functional differentiation along this axis.

The anatomical section explores the connectivity and neurochemical data relevant to this issue. The connectivity is divided into extrinsic (connections between the hippocampus and other structures), entorhinal cortex (connections between the EC and other structures and between EC and the hippocampus) and intrinsic (connections within the hippocampus). The entorhinal cortex, although strictly speaking, part of the hippocampal formation, is considered separately here because it constitutes the main cortical input into the hippocampus and as such contributes to the septotemporal segregation found within DG and CA.

Numerous in depth reviews on the anatomy of the hippocampus have been written (Amaral and Witter, 1995; Lopes da Silva et al, 1990; Witter et al, 1989; Swanson and Cowan, 1977). This chapter draws upon these reviews but presents the data in a rather different way: relevant functional data, which are usually ignored in purely anatomical reviews, will be included; and the extrinsic circuit will be discussed based on each of the structures that are connected to the hippocampus rather than on the particular hippocampal elements. With respect to the functional information, this will be somewhat unbalanced, the reason being that behavioural data corresponding to structures that are key to a possible functional differentiation along the longitudinal axis are discussed in more detail than those related to structures that do not seem, to date, to play an important role in this issue.

Little is known about the role of CA2, presubiculum and parasubiculum, all of which are parts of the hippocampal formation, and, for this reason, they are not considered. Cellular data of no relevance to the septotemporal segregation will also be omitted.

First, a quick but essential definition of terms that will be repeatedly used through the chapter. A constant feature through the hippocampal anatomy is that projections are segregated both along the transverse axis and along the longitudinal or septotemporal axis. In the transverse topography the projections can be limited to zones (proximal or distal) within the regions or elements (EC, DG, CA3, CA1 and subiculum) of the hippocampus. Proximal and distal mean closer to the preceding and the following element, respectively, according to the order given in the previous sentence. Thus, proximal and distal zones of CA3 correspond to the transverse zone closer to DG and CA1, respectively; while proximal and distal CA1 refers to the part of CA1 closer to CA3 and the subiculum. The septotemporal topography is defined in terms of levels (septal, splenial or temporal) within the longitudinal axis. Septal is the area that is more rostral, closer to the septal formation and temporal is the area that is more caudal, immersed in the temporal lobe. See Figure 4.1.

Functional data addressing the subject of a septotemporal gradient are divided in physiological and behavioural. Only studies published before 1993 (the first Moser et al. study) are included here. Studies published during the completion of the

experiments presented in Chapter 5 to 7 are discussed together with the results obtained in those chapters.

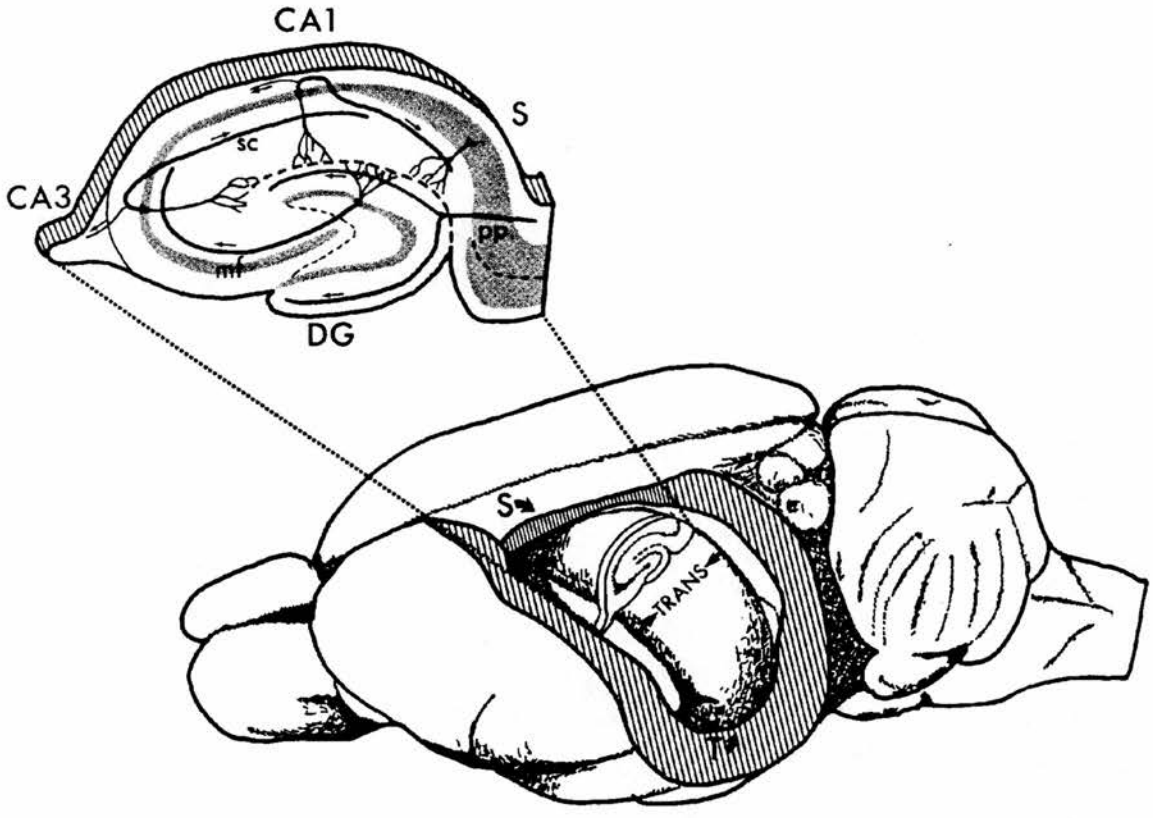


Figure 4.1: Nomenclature for the topography along the transverse and longitudinal (or septotemporal) axis of the hippocampus. Modified from Andersen et al. (1973).

4.1 The extrinsic circuit

The hippocampal formation receives both subcortical and cortical inputs, but not homogeneously along all the elements. DG and CA3 for example do not receive any cortical information (with the exception of EC inputs) and CA1, although rich in inputs of all kinds, is divided along the septotemporal axis by their nature. Outputs are more common to cortical than subcortical structures but they only arise from certain elements of the hippocampal formation: CA1, subiculum and EC. Connections with subcortical structures are mainly via the fimbria-fornix system, i.e. from rostral co-ordinates.

4.1.1 Subcortical connections

Figure 4.2 schematizes the data described in this section.

4.1.1.1 Basal Ganglia

The basal ganglia act as a relay station between the thalamus and the cortex and, as a result, they are involved in several motor functions. The nucleus accumbens (NAcc, ventral striatum), more specifically, is understood as a neural interface between limbic and motor systems and, accordingly, its role in contextual fear conditioning (Riedel et al., 1997) has been interpreted as mediation between hippocampal and motor functions. Although the NAcc is not necessary to learn a reference memory task in the watermaze (Thifault et al., 1998, but see Setlow and McGaugh, 1998), lesions to the structure result in slower learning rate as compared to control animals (Annett et al., 1989).

Proximal areas of the subiculum project to the nucleus accumbens and ventral claustrum. The septal area projects to the core of the nucleus accumbens and the temporal third projects to the shell as well as to the ventral claustrum (Groenewegen et al., 1987). The temporal level of CA1 also projects to the NAcc.

The effects of the temporal (Lipska et al., 1992), but not septal (Lipska et al., 1991), hippocampal projection to the NAcc are mediated by dopamine.

4.1.1.2 Basal Forebrain

This is a part of the limbic system comprised by the diagonal band of Broca (DBB) and the septum itself. The medial septum (MS) and DBB project to and the lateral septum (LS) receives inputs from the hippocampus. Although, it was traditionally believed that the LS sent projections to the MS, this is now known to be incorrect (Leranth et al., 1992). As an exception to the general pattern, the very temporal tip of CA and subiculum receive a weak projection from lateral septum.

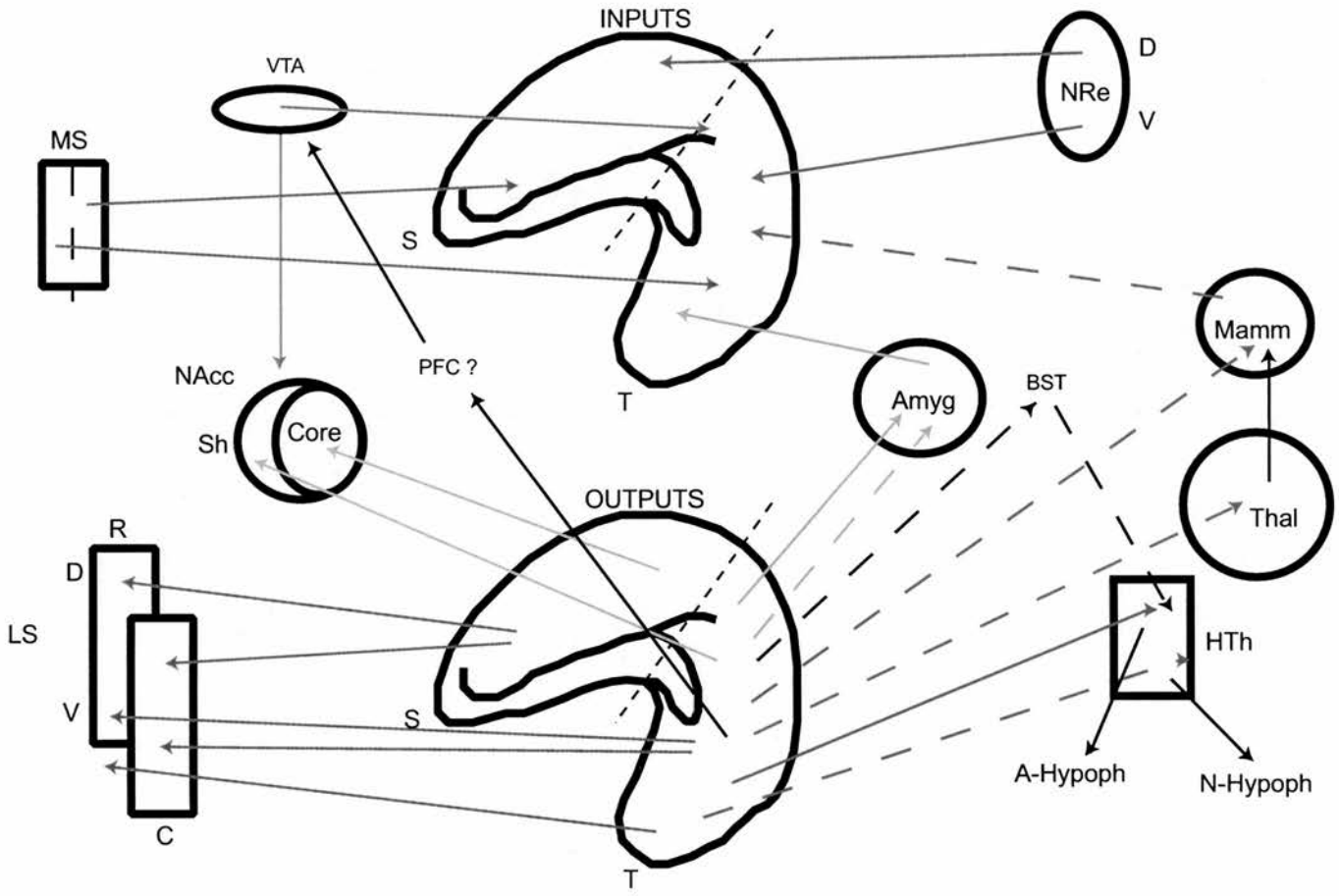


Figure 4.2: Subcortical inputs (top) and outputs along the septotemporal axis. S: septal; T: temporal; R: rostral; C: caudal; D: dorsal; V: ventral; MS: medial septum; VTA: ventral tegmental area; NAcc: nucleus Accumbens; Sh: shell; PFC: prefrontal cortex; LS: lateral septum; NRe: nucleus reuniens; Amyg: amygdala; BST: bed nucleus of the stria terminalis; Mamm: mammillary bodies; Thal: thalamus; HTh: hypothalamus; A- and N- Hypoph: adeno- and neuro- hypophisis. Dashed lines are used to represent those connections that affect the subiculum only.

As input

Lesions to the medial septum do not impair navigation in the watermaze (Torres et al., 1994; Compton et al., 1997; but see Hagan et al., 1988). They do, however, impair open-field activity and delayed non-matching to position (Torres et al., 1994). It has been suggested that these lesions disrupt attention (Voytko et al., 1994) or the capacity to use non stereotyped responses (Janis et al., 1994) rather than learning and memory processes. Others maintain that the medial septum is essential for hippocampal dependent mnemonic processes (Vertes and Kocsis, 1997). The septum is thought to act as a pacemaker and modulate hippocampal theta rhythm (Rawlins et al., 1979; Oka and Yoshida, 1985; Bland and Bland, 1986; Leung et al., 1994; Bland et al., 1999). Theta, however, can also be found in hippocampal slices that do not contain the septum (Konopacki et al., 1987) and hippocampal theta can be generated by brainstem nuclei (Vertes et al., 1993; Kocsis and Vertes, 1997) although it is suggested that this input travels via the medial septum (Oddie et al., 1996).

The septal projection includes the only cholinergic input received by the hippocampus, and more specifically glutamatergic cells (Alonso and Köhler, 1984). It is also a very important inhibitory projection terminating in several different kinds of hippocampal GABAergic interneurons (Miettinen and Freund, 1992). This inhibitory input is believed to play an important role in hippocampal theta rhythm (Toth et al., 1997). The projection travels mainly through the fimbria-fornix but a small component originating in the vertical DBB travels ventrally and enters the hippocampus from the temporal pole (Milner and Amaral, 1984).

MS and vertical DBB project to medial EC while horizontal DBB project to lateral EC (Milner and Amaral, 1984; Alonso and Köhler, 1984). Other areas of hippocampus (DG, CA3 and CA1) receive MS and DBB projections in a topographical manner (Meibach and Siegel, 1977a; Amaral and Kurtz, 1985; Yoshida and Oka, 1994). The septal part of the structure receives non-cholinergic inputs from the medial MS and cholinergic terminals (50 to 70% of the projection) from the DBB. The temporal parts of DG and CA receive cholinergic input from the lateral MS (30 to 50% of the fibres are cholinergic).

As output

Lesions to the lateral septum affect stereotyped behaviours and grooming (Lee et al., 1988). The lateral septum acts as a relay structure for inputs from the hippocampus to the hypothalamus (Risold and Swanson, 1996). These will be discussed in the section on the hypothalamus.

The projections from hippocampus proper and subiculum are topographically organized (Swanson and Cowan, 1977; Risold and Swanson, 1996). Septal and temporal hippocampal areas project to dorsal and ventral lateral septum respectively. CA3 projects mainly to caudal LS and within this projection, distal and proximal CA3 project to medial and lateral LS respectively. CA1 and subiculum project mainly to the rostral LS, the subicular projection originating in the proximal area. The very temporal tip of CA1 project to the ventral region of the LS.

4.1.1.3 Amygdala

Examples of modulation of hippocampus function by the amygdala have been widely reported. For example, although lesions to the amygdala do not generate impairments in the watermaze (Sutherland and McDonald, 1990), this structure modulates acquisition of this task by the hippocampus (Packard et al., 1994; Packard and Teather, 1998). Hippocampal LTP is also modulated by the basolateral and basomedial amygdala (Ikegaya et al., 1996 and 1997). However, the amygdala does more than modulate hippocampal function. It is known to be involved in fear conditioning (Davis, 1992; LeDoux, 1993; Campeau and Davis, 1995; Maren et al., 1996; Killcross et al., 1997; Maren, 1999), detection of stimuli of emotional relevance and development of appropriate responses (Rogan and LeDoux, 1996) and establishment of stimuli reward associations (Ehlers et al., 1998). That these two structures can act independently of each other is supported by the finding of a double dissociation of hippocampal and amygdalar function in memory reported by Bechara et al. (1995) in humans. Also, the amygdala and hippocampus are known to gate each others inputs to the nucleus accumbens (Mulder et al., 1997), and their role is found to be sequential in certain forms of memory (Izquierdo et al., 1997). Furthermore, the hippocampus can influence the amygdala. For example, information acquired by the

hippocampus influences amygdala dependent acquisition (McDonald and White, 1995) and hippocampal stimulation induces plasticity in the amygdala (Maren and Fanselow, 1995).

The basolateral nucleus of the amygdala is connected reciprocally with the temporal 1/3 of CA1, especially the distal part (van Groen and Wyss, 1990; Canteras et al., 1992a). The distal areas of CA1 project to medial medial entorhinal area (medial MEA), which in its most caudal part projects to septal DG. Adjacent CA1 also projects lightly to the posterior basomedial nucleus (Ottersen, 1982). The ventral subiculum, on the other hand, projects heavily to the posterior basomedial nucleus (Canteras and Swanson, 1992a), but faintly to the basolateral nucleus (Ottersen, 1982). Other nuclei of the amygdala (posterior basolateral, central, posterior, posterior cortical, intercalated and the nucleus of the lateral olfactory tract) are faintly innervated.

Posterior basomedial (Canteras et al., 1992a) and posterior cortical (Ottersen, 1982) amygdala project to proximal subiculum at all septotemporal levels.

The lateral and basolateral amygdala receive inputs from the temporal CA1, while the temporal subiculum projects mainly to the posterior basomedial nucleus. The latter receives projections from the lateral and posterior basolateral nuclei. The posterior basomedial nucleus, thus, becomes an integrator of cortical information. This nucleus projects to similar structures as the ventral subiculum (Canteras and Swanson, 1992a): the nucleus accumbens, the bed nucleus of the stria terminalis (BST), the ventromedial hypothalamus and the ventral premammillary nucleus. This means that BST nuclei receive information processed both by the hippocampus and the amygdala. The basomedial nuclei are also connected to the central nucleus of the amygdala. This nucleus is responsible for the amygdalar behavioural output, determined by the stimulus representations of each of the amygdalar nuclei (Pitkänen et al., 1997).

4.1.1.4 Thalamus.

Thalamic nuclei that project or receive projections from the hippocampus are midline ('non-specific') interlaminar nuclei in the anterior part of the telencephalon (Price, 1995). These nuclei have been implicated in spatial learning (Aggleton et al., 1995; Parker et al., 1997) and episodic memory (Aggleton and Brown, 1999).

In particular, projections from the nucleus reuniens terminate along the whole septotemporal extent, specially intermediate levels, of CA1 (Wouterlood et al., 1990 and Dolleman-van der Weel and Witter, 1996). The dorsal and ventral parts of the nucleus project to septal and temporal levels, respectively. The meaning of this topography is unknown to us. Different but intermingled population of cells in the nucleus reuniens (Dolleman-van der Weel and Witter, 1996) project to subiculum, with the difference that the septal and temporal tips of this region are devoid of thalamic terminals. The subiculum is also innervated by the parataenial and paraventricular nuclei.

Only the subiculum sends direct projections to the thalamus (Canteras and Swanson, 1992a; Meibach and Siegel, 1977b). However, the anterior thalamus receives indirect projections from the hippocampus through the mammillary body by means of the mammillothalamic tract. The thalamus in turn projects to the anterior cingulate cortex. These connections are all part of the original circuit proposed by Papez (1937) as the basic structure of the limbic system.

4.1.1.5 Hypothalamus and Mammillary Bodies

The hypothalamus is formed by a series of areas and nuclei whose limits, function and connectivity is not always straightforward (see Simerly, 1995). It is associated with the modulation of social, sexual and ingestive behaviours as well as stress responses. It is also responsible for different physiological reflexes, such as urine flow, milk ejection or uterine contraction, for general osmoregulatory responses such as termoregulation and metabolism, and for sleep. The hypothalamus acts as an interface between nervous and endocrine functions. The paraventricular and supraoptic nuclei innervate the neurohypophysis, where they release the hormones

vasopressin and oxitocine. The dorsomedial and ventromedial hypothalamic nuclei, on the other hand, synthesize hormone releasing factors that reach the adenohypophysis and activate the release of several hormones into the blood.

The mammillary bodies are located caudal to the hypothalamus and, as mentioned before, form part of the so called Papez circuit. They are strongly influenced by auditory and visual information and they are, also, associated with spatial memory (Neave et al., 1997). The dorsal premammillary nuclei acts as an interface between the mammillary body and the anterior nuclei of the hypothalamus (Canteras and Swanson, 1992b), the latter sending projections forward to the limbic system. The ventral premammillary nuclei receive inputs from sexually dimorphic areas in the forebrain such as the BST and the amygdala (Canteras et al., 1992b), that project mainly olfactory information. Feed back projections to these areas form part of a circuit that underlies certain aspects of sexual behaviour and physiology (Beltramino and Taleisnik, 1980). The supramammillary nucleus contains some dopaminergic cells and projects to telencephalon in general, excluding the striatum. Finally the tuberomammillary nucleus projects to many different areas in the brain from the telencephalon to brain stem nuclei. It has been suggested that this nucleus is involved in modulation of arousal and behavioural states although its projections to the paraventricular (PVN) and supraoptic (SON) nuclei also suggest an involvement in neuroendocrine functions (Ericson et al., 1987). All the areas mentioned receive inputs from the BST, the relevance of which will become clear below.

DG receives projections from the supramammillary region (Dent et al., 1983 and Vertes, 1992). CA2, although omitted in the rest of the Chapter, needs mention here because it is characterized by inputs from the supramammillary (Haglund et al, 1984) and tuberomammillary (Köhler et al., 1985) regions. The subiculum, particularly the temporal pole, receives substantial projections from the supramammillary area (Haglund et al., 1984) and the premammillary nucleus (Canteras et al., 1992b). Other scattered cells in the hypothalamic area raise a sizeable but diffuse projection to different regions of the hippocampus.

In terms of outputs, temporal levels of CA1 innervate anterior and dorsomedial hypothalamic areas (van Groen and Wyss, 1990 and Jay et al., 1989). The septal two thirds of the subiculum project to the mammillary nuclei (Canteras and Swanson, 1992a) and the temporal third, to the entire rostro-caudal extent of the hypothalamus, but mainly to the ventromedial hypothalamic nucleus (Canteras and Swanson, 1992a).

The hippocampus influences hypothalamic function also through indirect projections via the lateral septum (LS; Risold and Swanson, 1996). Different hippocampal fields terminate in different areas of the LS, which in turn project to different areas of the hypothalamus. CA3 projects to the caudal LS. CA1 and subiculum project to rostral LS which is associated with the medial hypothalamus (social behaviour). The topography of this projection is such that septal areas and temporal areas of CA1 and subiculum project to dorsorostral LS and ventrorostral LS which are associated with hypothalamic areas responsible for female and male behaviour respectively. The very ventral tip of CA1 and subiculum project to ventral LS areas connected to areas of hypothalamus associated with ingestive behaviour (Risold and Swanson, 1996).

The hippocampus also contains the highest brain levels of type I and II of glucocorticoid receptors (Herman et al., 1989). Glucocorticoids are released during stress as a result of activation of the hypothalamus-adenohypophysis axis. Chronic release of this type of hormone during severe stress can result in pyramidal cell damage. Conversely, absence of glucocorticoids as a result of adrenalectomy leads to degeneration of other neurons such as granular cells (Sloviter et al., 1989). This dual action is reflected in hippocampus-dependent functions such that these are first facilitated by increasing doses of glucocorticoids and then progressively impaired when the doses reach a certain threshold.

The hippocampus has an inhibitory effect over the hypothalamus. Hippocampal stimulation inhibits median eminence-projecting neurons in the PVN (Saphier and Feldman, 1987), reduces plasma corticoesteroid levels, and inhibits stress-induced costicosterone secretion (Dunn and Orr, 1984). Hippocampal ablation has opposite effects (Feldman and Conforti 1980; Herman et al., 1989; Sapolsky et al., 1991). The

bed nucleus of the stria terminalis might act as an intermediary in these effects (Cullinan et al., 1993). The ventral subiculum does activate the neurosecretory motorneurons directly (Swanson et al., 1987) but might be able to do so through projections terminating in the BST. GABAergic cells from the BST then project to the PVN. There is some evidence for a direct projection from the subiculum to the PVN (Kiss et al., 1983) but the inhibitory effects could not be direct because hippocampal outputs are excitatory. There are other structures that receive projections from the subiculum, have a high content of GABA cells and project to the PVN, but there is evidence that some of these structures do not mediate the inhibitory effect (Herman et al., 1990 and 1992).

4.1.1.6 Brain Stem

The hippocampus receives diffuse but functionally significant projections from the locus coeruleus (noradrenergic), the raphé (serotoninergic) and the ventral tegmental area (dopaminergic).

Noradrenergic locus coeruleus inputs are distributed along the whole hippocampus (Haring and Davis, 1983) , although only lightly in the subiculum.

Different nuclei in the raphé formation project to DG (mainly nonserotoninergic fibres; Köhler and Steinbusch, 1982; Montone et al., 1988), to the CA3 (serotoninergic, Swanson et al., 1987) and lightly to the subiculum. These projections arise from the median raphé (Vertes et al., 1999).

The ventral tegmental area (VTA) projection is not exclusively dopaminergic. It projects to the dentate gyrus diffusely (Swanson, 1982), the dorsal and ventral subiculum and adjacent CA1 regions (Gasbarri et al., 1994). They are very ramified projections whose terminal field matches the areas of origin of the projection to the nucleus accumbens (Groenewegen et al., 1987, Kelley and Domesick, 1982). The VTA projection to the nucleus accumbens terminates in the same areas as the projections from the hippocampal formation (Sesack and Pickel, 1990 and Totterdell and Smith, 1989). The VTA may then modulate the link between the limbic system and the basal ganglia. In fact, the already mentioned effect of hippocampal activity

over dopamine levels in NAcc, could be mediated by the ventral tegmental area (VTA). The hippocampal input to the VTA that would mediate this effect, however, is still unknown. The prefrontal cortex, which receives projections from the hippocampus (Swanson et al., 1981; Jay and Witter, 1991) and projects to the VTA (Sesack and Pickel, 1990; Rosetti et al., 1998), has been proposed for this role (Legault et al., 2000).

4.1.1.7 Conclusions

The hippocampus is rich in subcortical projections. Two things are apparent in Figure 4.2: first, projections with a septotemporal topography or limited to a particular septotemporal level are the common rule and second, there are more efferents than afferents and these tend to originate from the temporal part of the hippocampus. Thus, it seems that the hippocampus exerts influence over a greater number of subcortical structures than that which projects to the hippocampus. CA1 and subiculum areas play a stronger role in subcortical connectivity. In fact, with the exception of connections with the basal ganglia and septal formation, DG and CA3 are devoid of it.

The temporal half of the hippocampus is differentially associated with the amygdala and the hypothalamus. This fact will be further discussed in relation to the behavioural data in Chapter 5. Other projections are segregated to different levels of the hippocampus but the meaning of this topography is in many cases unknown. This is the case of the segregation of the dorsal and ventral reuniens or the medial medial and lateral medial septum to septal and temporal hippocampus respectively. Because little is known about the topography of those structures themselves little can be concluded about the pattern of their connections with hippocampus.

The lateral septum, the mammillary bodies and the BST act as relay stations to other structures that are influenced by the hippocampus such as the hypothalamus and thalamus. This highlights the importance of indirect connections and their relevance in maintaining the septotemporal topography. The role of the lateral setpum on mediating the influence that different levels of the hippocampus exert over different hypothalamic areas is but one example.

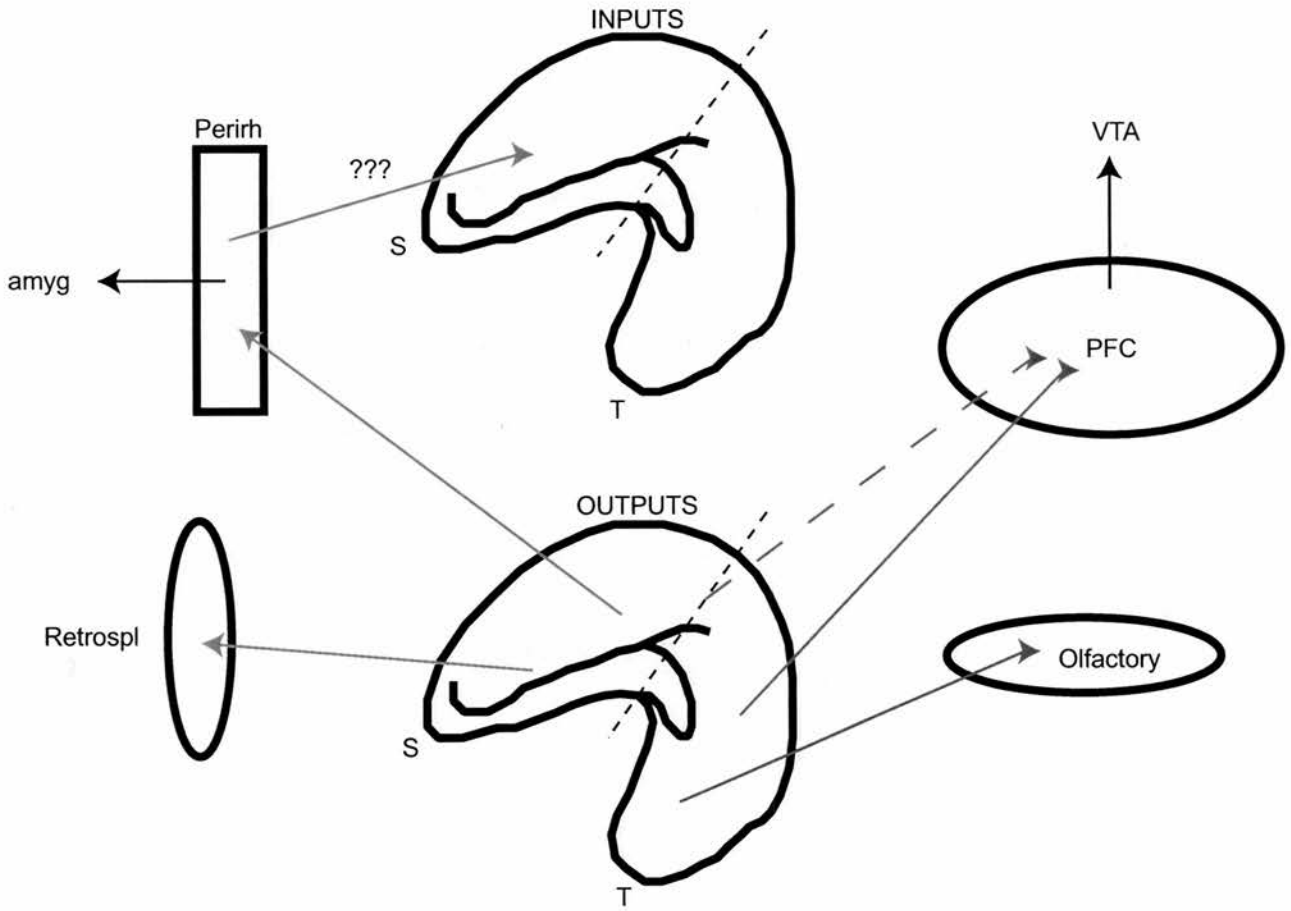


Figure 4.3: Cortical inputs (top) and outputs along the septotemporal axis. S: septal; T: temporal; Perirh: perirhinal cortex; amyg: amygdala; Retrospl: retrosplenial cortex; VTA: ventral tegmental area; PFC: prefrontal cortex; Dashed lines are used to represent those connections that affect the subiculum only.

4.1.2 Cortical connections

These are illustrated in Figure 4.3.

4.1.2.1 Perirhinal and Postrhinal cortices.

The perirhinal cortex is adjacent to the entorhinal cortex rostrolaterally. It is constituted by areas 35 and 36, the former being the more medial. It is known to be involved in some learning and memory processes (see Suzuki, 1996 for review), among which are most forms of object recognition (Meunier et al., 1993; Ennaceur et al., 1996; Wan et al., 1999; Murray and Bussey, 2000), and cued (Rosen et al., 1992; Campeau and Davis, 1995) and contextual (Corodimas and LeDoux, 1995; but see Phillips and LeDoux, 1995) fear conditioning. The perirhinal cortex is believed to be involved in certain forms of spatial memory by some (Wiig and Bilkey, 1994a and b; Nagahara et al., 1995; Liu and Bilkey, 1998). Others however have found evidence of the contrary (Aggleton et al., 1997; Bussey et al., 1999; Wan et al., 1999). The postrhinal cortex (parahippocampal in the monkey) is situated more caudally than perirhinal cortex. Little is known about the function of the postrhinal cortex in the rat so far. Interest has, however, increased in the last few years.

Both perirhinal and postrhinal cortices project mainly to the EC and constitute the main cortical inputs into the hippocampal formation. The reason to include them here rather than in the entorhinal cortex section is that perirhinal cortex is connected with CA1 and subiculum and, as the postrhinal cortex is very closely related with the perirhinal, it is more appropriate to describe them together.

The connection with these rhinal cortices cannot be fully understood without describing the general pattern of connectivity of these structures themselves. What follows is a brief overview of both cortical and subcortical connections.

Cortical projections

See Figure 4.4 for illustration of the text below.

Projections arise from both unimodal and polymodal associational areas. In the rat perirhinal cortex unimodal projections originate mainly in olfactory associational areas (terminating in area 35), but other associational inputs, such as auditory and somatosensory, are known (terminating in area 36).

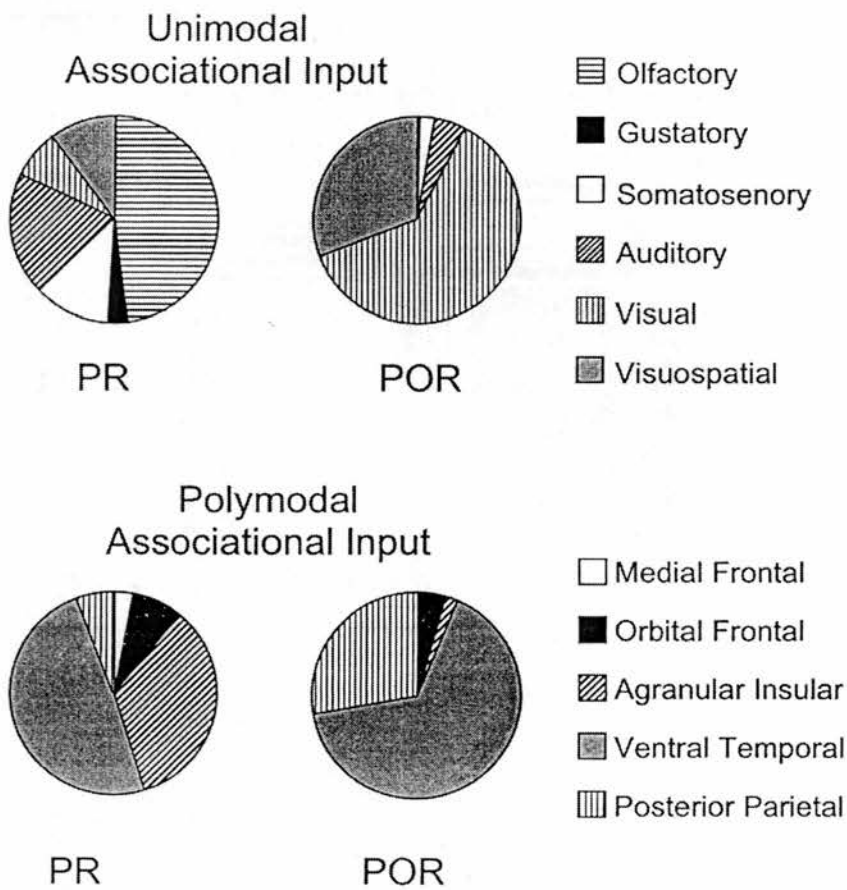


Figure 4.4: Cortical inputs to the perirhinal and postrhinal cortices, represented in pies by their relative abundance. PR: perirhinal cortex; POR: postrhinal cortex. From Burwell, 2000.

Postrhinal cortex, however, receives almost exclusively visual and visuospatial associational inputs, although, a small auditory component and a very weak input from the remaining modalities are also known (Burwell et al., 1995). Polymodal associational inputs (Deacon et al., 1983, in the rat; Room and Groenewegen, 1986a, in the cat; see Burwell, 2000 for review) originate in medial and orbital frontal, agranular insular, ventral temporal and posterior parietal cortices. Those originating in the ventral temporal association cortex, are by far the heaviest and are organized such that the full rostrocaudal extent (Te) projects to the perirhinal cortex and only the most caudal portion (associated with visual inputs), to postrhinal cortex.

Subcortical projections to perirhinal cortex.

These arise from claustrum, septal nuclei, amygdaloid complex, thalamus and hypothalamus, raphé and locus coeruleus (Deacon et al., 1983, in the rat ; Room and Groenewegen, 1986b, in the cat).

According to the cortical and subcortical pattern of connectivity, it is suggested (Burwell, 2000) that the processing stream including the perirhinal cortex might be involved in 'attentional processing of behaviourally relevant stimuli', while that containing the postrhinal cortex might be involved in 'attentional orienting toward spatially relevant stimuli'.

Direct projections to the CA1 area have been described (Suzuki and Amaral, 1990; Liu and Bilkey, 1996 and 1997; see also Kosel et al., 1983 and Deacon et al., 1983). Canning and Leung (1997), however, claim that this projection does not exist as such and that it is fibres that originate somewhere else but traverse through the perirhinal cortex that reach CA1. Liu and Bilkey (1997) also report a direct projection to DG (see also Ruth et al., 1988; Witter et al., 1989) but this is somewhat controversial (Kosel et al., 1983; McIntyre et al., 1996).

Septal levels of CA1 and the subiculum send a sizeable projection to perirhinal cortex (van Groen and Wyss, 1990).

4.1.2.2 Olfactory cortical areas

Temporal levels of CA1 project to olfactory areas (van Groen and Wyss, 1990).

4.1.2.3 Prefrontal cortex

The prefrontal cortex (PFC) is constituted by the anterior cingulate , agranular insular, orbitofrontal cortices in the rat and for some also by infralimbic cortex. The PFC is associated with attention, planning and behavioural flexibility (Dias et al., 1996), and working memory (Goldman-Rakic, 1990). Lesions to the cingulate cortex produce a deficit in spatial reversal (see Kolb, 1990 for review).

Subiculum projects to orbitofrontal and weakly to anterior cingulate (Swanson and Cowan, 1977). These projections originate from the proximal subiculum. Temporal CA1 projects to infralimbic cortex (Swanson, 1981), while sending collaterals to EC.

4.1.2.4 Retrosplenial cortex.

Also known as posterior cingulate.

The septal two thirds of distal areas of the subiculum and the septal CA1 project to this part of cortex.

4.1.2.5 Conclusions

Only CA1 and subiculum have connections with cortical structures and these are almost exclusively output projections, with the exception of the controversial perirhinal input. CA1 efferents are clearly segregated along the longitudinal axis with the septal third terminating in perirhinal and retrosplenial cortex and the temporal third terminating in olfactory and prefrontal cortical areas.

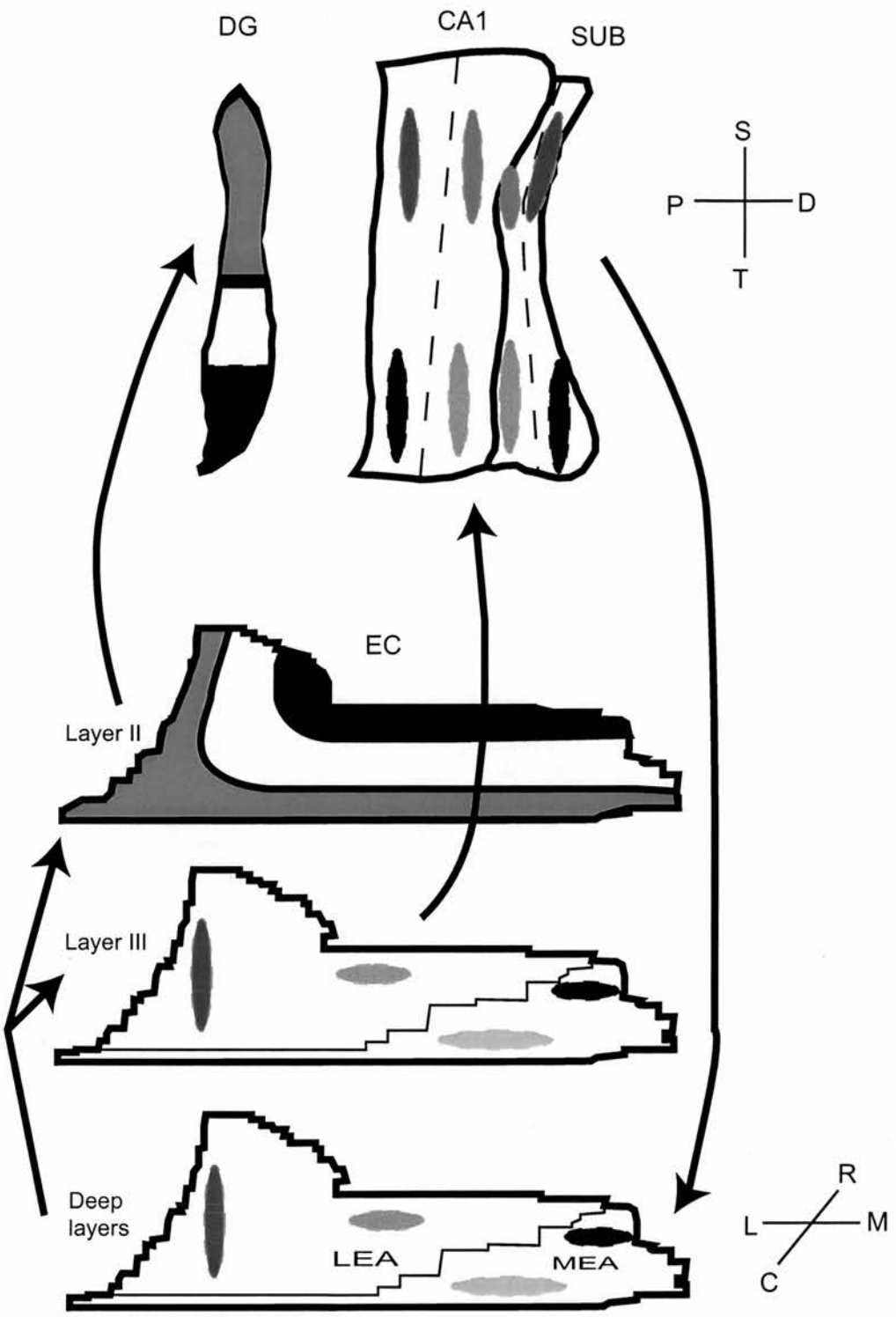


Figure 4.5: Topography of the connections between entorhinal cortex (EC) and the hippocampus (dentate gyrus, DG; CA1; and subiculum, SUB) represented over flat maps of the different areas. CA1 and SUB connections do not result in clear bands in the EC and, for this reason, are represented as coloured areas.
S: septal; T: temporal; P: proximal; D: distal; R: rostral; C: caudal;
L: lateral; M: medial.

4.2 The entorhinal cortex

4.2.1 Connections with the rest of the hippocampal formation.

See Figure 4.5 for illustration of the text.

4.2.1.1 Projections from the Entorhinal Cortex to the DG and CA3.

This projection is known as the perforant path because of the way it traverses the subiculum, after travelling in the angular bundle. It terminates at all the different septo-temporal levels in the hippocampus but has long been known to have a specific topography. The lateromedial axis of the entorhinal cortex projects to the septotemporal axis of the dentate gyrus (Ruth et al., 1982; Witter and Groenewegen, 1984; van Groen and Lopes da Silva, 1985; Witter, 1993; Dolorfo and Amaral, 1998a). It arises from the superficial layers of the entorhinal cortex, concretely layer II (Steward and Scoville, 1976; Witter, 1993).

To save the reader from the tedium of subtle differences between the published studies, the work of Dolorfo and Amaral's work (1998a) describing the topography of this projection in the rat will be presented in detail. The EC can be divided into three bands. The most external band, in the shape of a boomerang, comprises the most lateral and caudal parts of the EC. This band projects to the septal half of the dentate gyrus. The intermediate band, also in the shape of a boomerang, medial to the first band, projects to the third quarter of the dentate gyrus starting from the septal pole. Finally, the most medial band, comprising the more rostral and medial area of the EC, projects to the more temporal quarter of the dentate gyrus. Each of the three projections is both completely convergent and completely divergent. Any cell in any band projects to the full extension of its corresponding band in DG and any cell in a specific part of the dentate gyrus receives projections from the full extension of the corresponding band in the EC. Van Groen and Lopes da Silva (1985) described an identical projection segregation on the basis of electrophysiological studies in the cat.

Within the EC, cells in each band are connected only with cells in the same band (Dolorfo and Amaral, 1998b). This means that cortical or subcortical information that reaches one of the bands of the EC is not transmitted to the other bands in the structure or, subsequently, to different areas of the DG other than those to which the band projects to. See Figure 4.6.

The three DG-projecting EC bands are independent of the classical distinction between lateral (LEA) and medial (MEA) entorhinal areas (see Fig. 4.7), which is based on morphological features. LEA projects to the outer third of the DG molecular layer while MEA projects to the middle third. Previous works have described projections between LEA and MEA. Witter and colleagues (1986) affirm that the dorsal part of the LEA receives information from MEA in studies done in the cat. As the three DG-projecting EC bands include parts of LEA and MEA and connectivity within these areas is profuse (Dolorfo and Amaral, 1998b), this is not surprising. Some of the projections of the perforant path send collaterals to CA3. These collaterals maintain the topography of the EC to DG projection.

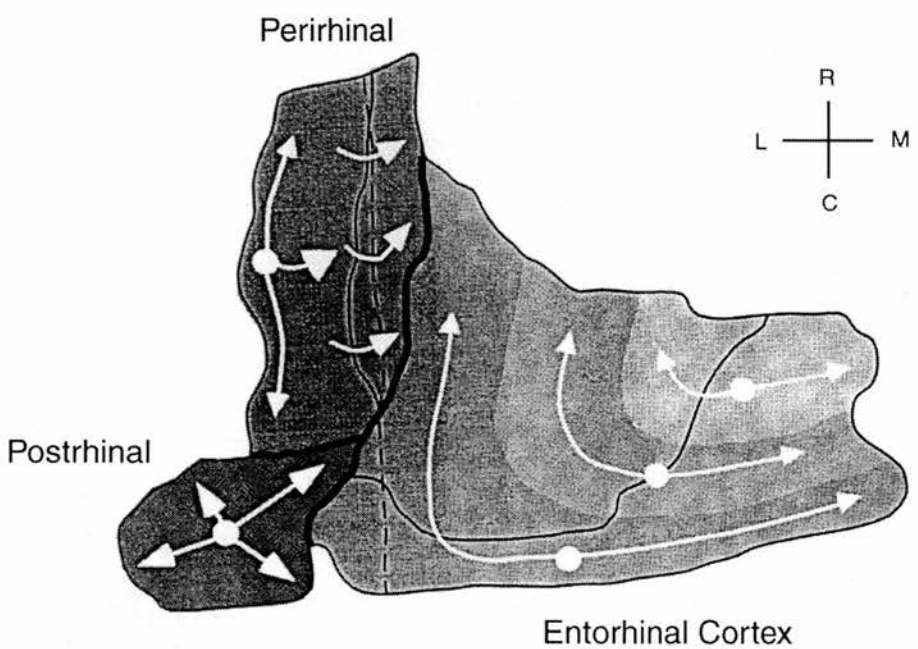


Figure 4.6: Intrinsic EC connectivity is profuse within the DG-projecting bands but not across them. From Burwell, 2000.

Cortical and subcortical EC connections to superficial and deep layers

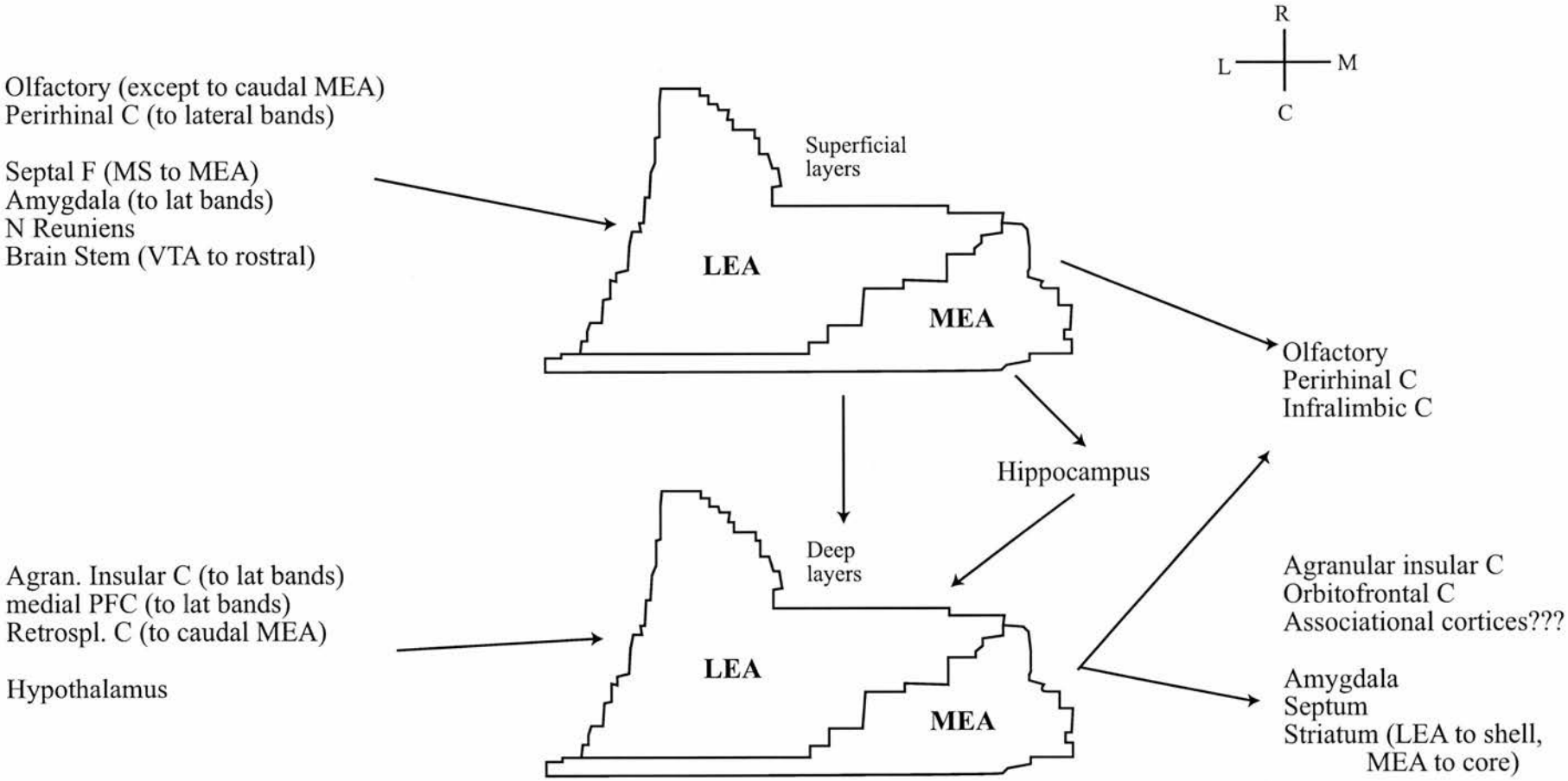


Figure 4.7: Cortical and subcortical connections with superficial and deep EC layers. Topography in brackets. R: rostral; C: caudal; L: lateral; M: medial: F: formation; MS: medial septum; N: nucleus; VTA: ventral tegmental area; PFC: prefrontal cortex.

4.2.1.2 Projection from the EC to CA1 and subiculum.

This projection originates in layer III. Its topography is identical, point by point, to that of the projection CA1 and subiculum send back to the EC.

4.2.1.3 Projections from CA1 and subiculum to Entorhinal Cortex.

Septal and temporal, but not splenial, levels of CA1 project to the entorhinal cortex (van Groen and Wyss, 1990). Different points along the transverse axis of CA1 and subiculum project to LEA or MEA and different septotemporal levels project to lateral or medial EC. Specifically, distal CA1 and proximal subiculum project to LEA such that septal and temporal levels project to lateral and medial LEA respectively. Conversely, proximal CA1 and distal subiculum project to MEA, such that septal and temporal levels project to lateral and medial MEA. All these fibres terminate in layer IV of EC with the exception of fibres originating in temporal levels of proximal CA1 and distal subiculum that terminate in layers I-III instead (Witter, 1993).

4.2.2 Subcortical connections

See Figure 4.7 for illustration of subcortical and cortical EC connections.

These are divided in two groups: those that project to superficial layers of EC and, thus, to layers that send projections to the hippocampus; and those that project to deep or input layers.

Among the first are inputs from the septal formation, amygdala, thalamus and brain stem. Among the second are inputs from hypothalamus and outputs to striatum, septum and amygdala.

Lateral and medial parts of EC project to the nucleus accumbens and terminate in rostrolateral (or shell) and caudomedial (or core) divisions, respectively. This means

that the subicular and EC areas that are interconnected project to different divisions of the nucleus accumbens.

The medial septum and diagonal band of Broca project to superficial layers of EC. The lateral septum, and to a lesser extent the medial septum, receive projections from, mainly, deep layers of this structure (Room and Groenewegen, 1986b). Thus, the septal formation influences layers that project to the hippocampus and is influenced by cells receiving inputs from the hippocampus.

The lateral and basolateral nuclei of the amygdala are reciprocally connected to ventrolateral EC (Krettek and Price, 1977; Wyss, 1981; Swanson and Köhler, 1986). This part of EC projects to the septal hippocampus, which is not, itself, connected with the amygdala. Kindling of the amygdala results in mossy fibre reorganization along the whole septotemporal extent of the structure (Cavazos et al., 1992). Also, there is evidence that the amygdala modulates the effects of various drugs injected in the dorsal hippocampus (Roozendaal and McGaugh, 1997). It is possible that these effects are mediated by this EC efferent.

Thalamic terminals are also found in superficial layers of EC, mainly from nucleus reuniens (a different cell population than the one projecting to CA1 or subiculum).

Deep layers of the EC receive a diffuse projection from the supramammillary area (Haglund et al., 1984), the tuberomammillary nucleus (Köhler et al., 1984) and the lateral hypothalamus (Köhler et al., 1985).

In the brain stem, different raphe nuclei project diffusely to EC (superficial or output layers). The VTA, also terminating in superficial layers, projects to rostrolateral LEA (Swanson, 1982) and, thus, to areas projecting to septal DG.

4.2.3 Cortical connections

The perirhinal cortex projects to layers I and III of lateral LEA and, to a lesser extent, lateral MEA (Burwell and Amaral, 1998a). These projections originate in superficial layers of the perirhinal cortex and cover the whole longitudinal extend (Witter and

Groenewegen, 1986 in the cat). Transverse connections are more restricted. Therefore while all parts of perirhinal cortex project between each other, projections to entorhinal cortex reach mainly the lateral band of this structure (Burwell and Amaral, 1998b). See Fig. 4.8. Here they will be influenced by heavier olfactory inputs than those received by the perirhinal cortex itself. Both superficial and deep layers send back projections to the perirhinal cortex.

Postrhinal cortex projects mainly to lateral and intermediate parts of LEA and lateral MEA (Burwell and Amaral, 1998b), again both areas of EC that project to septal parts of DG.

The whole of the EC, except caudal MEA, receives projections from olfactory areas (van Groen and Wyss, 1990).

The lateral entorhinal cortex projects to the whole of the prefrontal cortex in the rat (agranular insular cortex, cingulate areas and prelimbic areas; Jay et al., 1989). Deep layers of entorhinal cortex project to infralimbic cortex while both superficial and deep layers project to orbitofrontal and agranular insular. EC receives projections

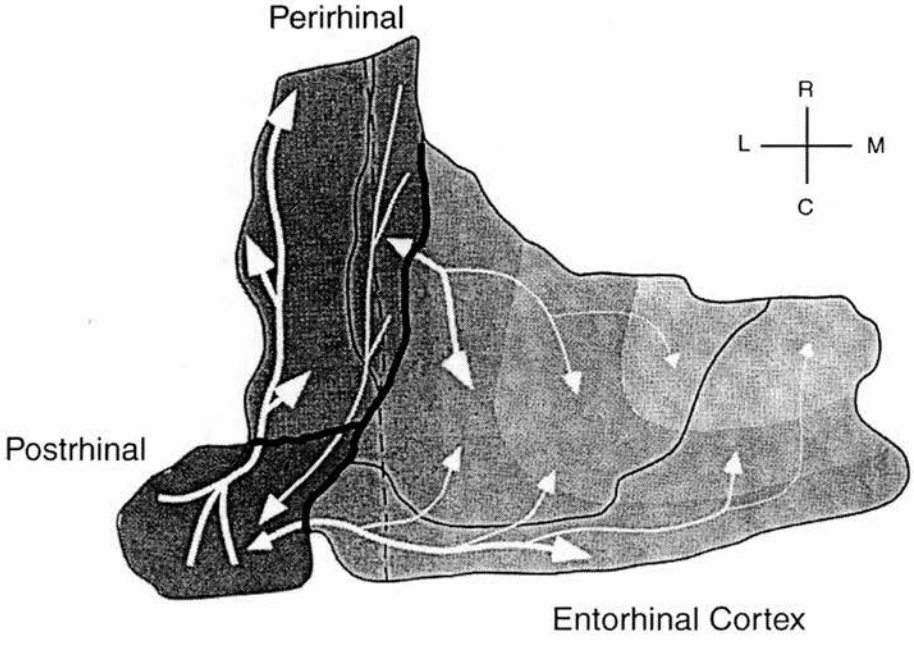


Figure 4.8: Topography of perirhinal and postrhinal inputs to EC, with respect to the DG-projecting EC bands. From Burwell, 2000.

from the agranular insular cortex (Reep and Winans, 1982), anterior cingulate, prelimbic, infralimbic and orbitofrontal cortices (Room and Groenewegen, 1986a), which terminate in deep layers.

Medial entorhinal cortex projects to the ventral retrosplenial cortex (Witter and Groenewegen, 1986).

Whether the EC send projections to unimodal and polymodal associational cortices is an unresolved issue. It has not been established whether some of the efferents to these cortices originate in lateral EC or medial perirhinal cortex. The latter is known to send a sizeable projection to associational areas.

Subcortical and cortical inputs to the EC, thus, generally terminate in areas of EC projecting to septal hippocampus. Conversely, most outputs originate in areas of EC receiving inputs from hippocampus.

4.3 The intrinsic circuit

While hippocampal projections to and from other structures, including the EC, show, in general, a strong septotemporal topography, the intrinsic circuit has an homogeneous appearance along the longitudinal axis. In fact, if one were to straighten the hippocampus along the longitudinal axis and make transverse cuts, these would look identical in terms of intrinsic organization whether taken from the septal or the temporal halves. Additionally, electrophysiological data suggest that excitatory pathways are organized in multiple parallel lamellas (Andersen et al., 1971) perpendicular to the longitudinal axis and of limited extent along this axis. Tamamaki and Nojyo (1991) later found that the angle of the lamella is not necessarily perpendicular to the longitudinal axis and that it varies depending on which particular projection is being considered. See Figure 4.9.

Such a system, although homogeneous along the longitudinal axis could maintain the septotemporal segregation created by the extrinsic circuit. However, as pointed out in Amaral and Witter (1995), when the septotemporal extent of some of the intrinsic

projections is taken into account, it becomes apparent that a lamellar organization of excitatory pathways is, although accurate, not a complete account. What follows is a description of the intrinsic circuit in relation to the septotemporal axis.

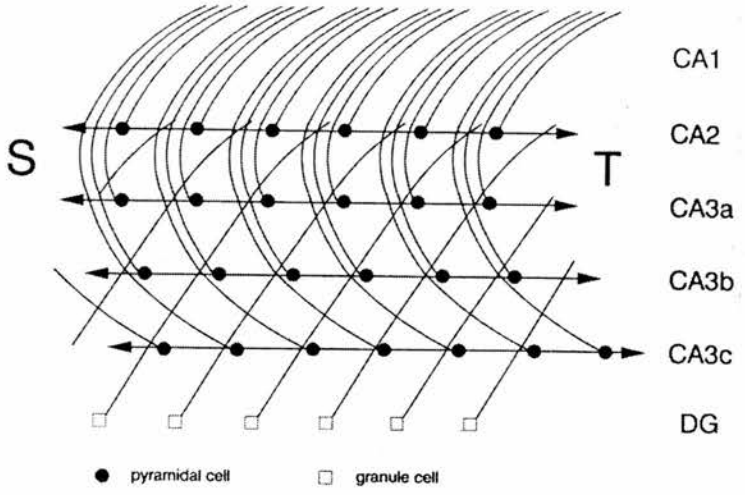


Figure 4.9: Lamellar organization of intrinsic hippocampal connections. The angle of the lamella is different depending on the projection. From Tamamaki and Nojyo, 1991.

4.3.1 Projections from Dentate Gyrus to CA3.

This projection is known as the mossy fibre projection. Its name is due to the morphology of the dentate gyrus terminals in the pyramidal layer of CA3 and to the excrecences developed by these pyramidal cells to synapse with the dentate gyrus boutons. The projection stays in a more or less transverse section of the hippocampus, i.e. within the lamella. One point of the dentate gyrus will project to the whole mediolateral extent of CA3 at the same septo-temporal level.

Despite this transverse organization, different aspects of the mossy fibre projection are segregated along the septotemporal axis. Gaarskjaer (1978) found evidence that, in the pyramidal layer, more area was occupied by boutons in the septal than in the temporal level of CA3. The cells were more widely spaced and the boutons were deeper septally. Also, in the granular layer, the length of the dendritic tree and the number of cells was bigger septally. The ratio of number of granular cells versus number of pyramidal cell (GC/PC) equals ten in the septal tip, decreasing linearly towards the temporal tip where it is one. Because of the transverse character of the

mossy fibre projection, GC/PC is equivalent to saying innervating / innervated. This means that there are ten times more innervating cells per innervated cells septally than temporally. According to Amaral and Witter (1995) there are 6 times more granular cells in the septal DG than in the temporal DG and there are 3 times more pyramidal cells temporally than septally. Although these numeric values somehow do not agree with those for the GC/PC ratio given above, the general trend is in both cases the same.

The mossy fibre gives collaterals that innervate the basket cells of the dentate gyrus. These in turn inhibit the granular cells that originated the projection and granular cells at neighbouring septotemporal levels. See Figure 4.10.

There are 40 granular cells per basket cell in the septal level while 220 granular cells per basket cell in the temporal level (Seress and Pokorny, 1981). In normal rats the mossy fibre projects to the basket cells of the supragranular layer only in the temporal tip (Cavazos et al., 1992). The fact that there are five times more granular cells per basket cell in the temporal dentate gyrus could be interpreted as there being less inhibition in the temporal dentate gyrus. This would, however, depend on the characteristics of the projections of the septal and temporal basket cells to granular cells. Another factor is the activity pattern of the granular cells which activate those basket cells. It is known for example that, in the cat, the projections to septal dentate gyrus from entorhinal cortex are of faster transmission than those that project to the temporal part of dentate gyrus (van Groen and Lopes da Silva, 1985). This would affect the influence that the basket cells would have over the granular cells.

Li and collaborators (1994) found that the proximal part of CA3 projects back to the granule cells. This constitutes the only exception known to the unidirectional nature of the connectivity between elements of the hippocampal formation. Their finding is based on *in vivo* intracellular labelling study on ten CA3 pyramidal cells. Three out of seven cells in dorsal CA3 and three out of three cells in ventral CA3, projected to DG. Although the number of cells seems too small to conclude anything they argue this might have a relevance to regional sensitivity to epilepsy in human

hippocampus. Amaral and Witter (1995) argue that the only projection from CA3 to DG terminates in the cells of the hilus not in the granular cells.

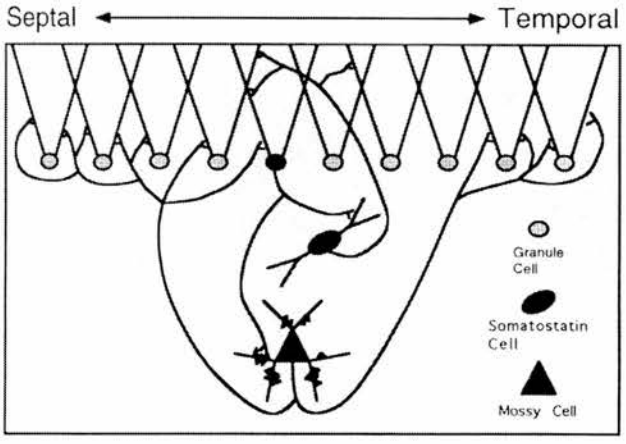


Figure 4.10: Basket cell inhibition of granular cells along the septotemporal axis. From Amaral and Witter, 1995.

4.3.2 CA3 associational projections.

CA3 pyramidal cells output results in collaterals terminating in CA2, CA1, lateral septum and CA3 itself. CA3 cells in proximal areas project only to other CA3 cells located in proximal areas in the same transverse axis or in septal or temporal neighbouring lamellas. CA3 cells in middle or distal areas on the other hand project to the whole transverse extent of CA3 in the same transverse axis but also along a fairly broad septotemporal extent (Ishizuka et al., 1990).

For the first time, information segregated along the septotemporal axis is mixed by means of projections that terminate over an extent of the axis that is broader than that covered by the bands determined by EC. In fact the CA3 associational system was believed to be the main longitudinal projection within the hippocampus (Finch et al., 1983; Amaral and Witter, 1989; Tamamaki and Nojyo, 1991; Li et al., 1994), although it is recognized that the longitudinal extent is narrower in temporal levels than in septal levels (Swanson et al., 1978).

4.3.3 Projection from CA3 to CA1.

This projection, known as Schaffer collateral, has a proximodistal to distoproximal topography, i.e. the proximal and distal portions of CA3 project to distal and proximal CA1, respectively. Traditionally it was thought to be limited to the transverse axis of the hippocampus (Tamamaki and Nojyo, 1991) but it is now known to have a very broad septotemporal extension. Ishizuka et al. (1990) presented evidence that proximal CA3 projects to all the septotemporal axis of CA1 but more densely to the septal pole. Distal parts of CA3, on the other hand, project to the same and to more temporal levels of CA1, the termination being heavier further temporally. See Figure 4.11.

Tamamaki et al. (1988) revealed that CA3 projections can distribute along as much as 75% of CA1 septotemporal axis. CA3 pyramidal cells project to stratum oriens and stratum radiatum of CA1 (two layers of the CA1 element), the density of the terminals in each stratum being different at different levels of the hippocampus (Ishizuka et al. 1990). Regardless of the transverse position of CA3 cells, there are more projections to stratum oriens in the septal CA1 and more to the stratum radiatum in temporal CA1 (Li et al., 1994).

4.3.4 Projections from CA1 to subiculum.

Originally thought to be restricted to a transverse level, cells in CA1 send projections that cover approximately one third of the subicular longitudinal axis (Amaral and Witter, 1995) with the septal fibres tending to move caudally before terminating in the subiculum (Swanson et al., 1978). The proximal and distal thirds of CA1 project to the distal and proximal thirds of the subiculum respectively. Middle CA1 third terminates in middle third of the subiculum. This pattern generates three columns of termination in the transverse axis of the subiculum. The septal CA1 projects to the contralateral subiculum, not so the splenial or temporal CA1 (van Groen and Wyss, 1990). See Figure 4.12.

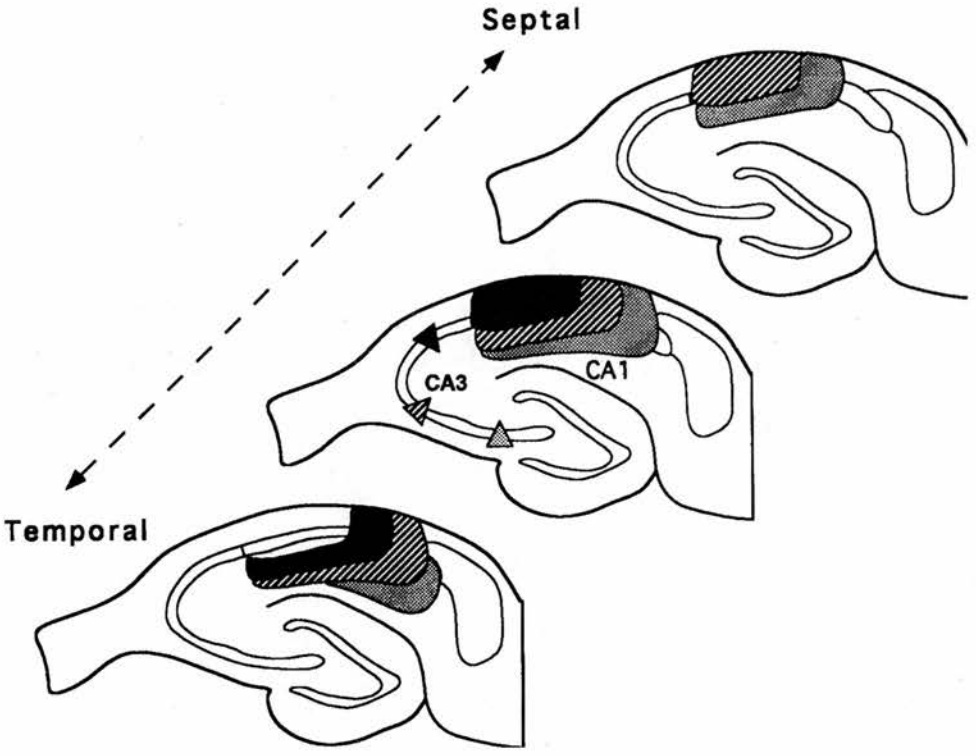


Figure 4.11: Transverse and septotemporal topography of the CA3 projection to CA1. Triangles represent the site of origin in CA3 and shaded areas represent the corresponding areas of termination in CA1. From Amaral and Witter, 1995.

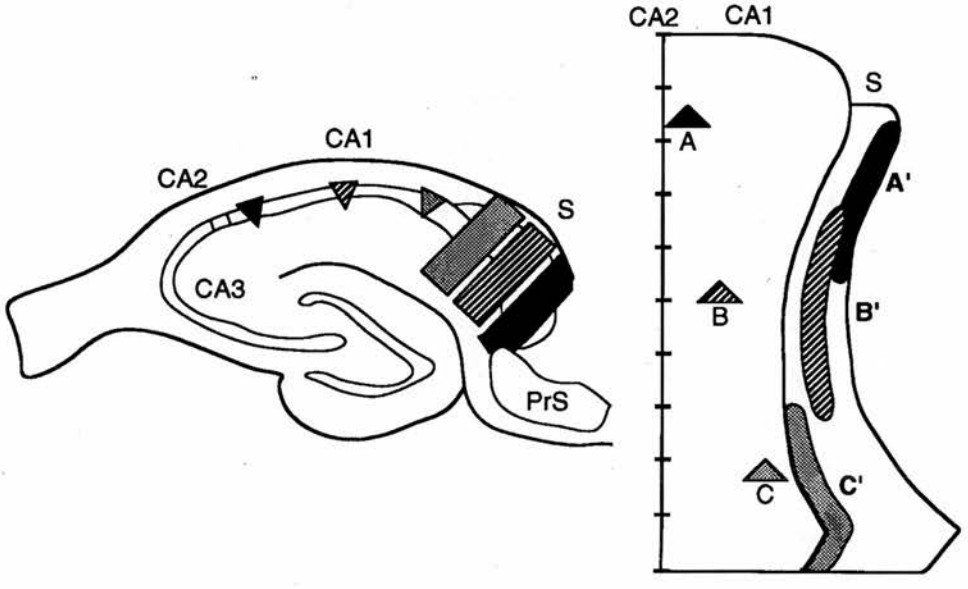


Figure 4.12: Transverse and septotemporal topography of the CA1 projection to subiculum (SUB). Triangles represent the site of origin in CA1 and shaded areas represent the corresponding areas of termination in the subiculum. From Amaral and Witter, 1995.

4.4 Overview of the extrinsic and intrinsic circuits: conclusions.

Hippocampal internal projections are not always restricted to the lamella and, although limited within particular areas of the septotemporal axis, the boundaries of these areas generally cross over the boundaries of areas determined both by the extrinsic circuit and by the perforant path projection. The DG projection to CA3 is, nonetheless, restricted to narrow septotemporal levels. The result is that the segregation of inputs created by the perforant path is maintained in the projection from DG to CA3.

When the EC is included in the picture, and as described in Chapter 1, information flow within the hippocampal formation appears to be partially parallel as well as serial. See Figure 1.2. The possibilities of such a circuit are numerous.

For example, activity in a particular area of the EC can reach CA1 via a direct projection, via a relay on CA3, or through a trisynaptic input via DG and CA3. A direct input will maintain the original septotemporal topography, however, an input travelling through CA3 might have lost that segregation by means of the CA3 associational inputs. Thus, activity in the EC might reach CA1 in three different states (processed via DG and/or CA3 or not processed), at the same or different septotemporal levels and at different times (a direct input will be faster than an indirect input). This organization is recognized as being key for hippocampal function by numerous computational studies.

What are the consequences of an intrinsic circuit that extends along the septotemporal axis for the segregation created along this axis by extrinsic and EC projections? Considering one of the three EC bands that projects to different DG levels, inputs from this band will reach CA3 without losing the segregation. However, once in CA3, both CA3 to CA3 associational connections and CA3 to CA1 outputs will transmit activity to septotemporal levels that are outside the scope of the original bands. See Figure 4.13. For example, activity in septal CA3 will be transmitted mainly to septal distal CA1 and to more temporal and proximal levels of CA1. The more temporal activity is in CA3, the more temporally it will appear in

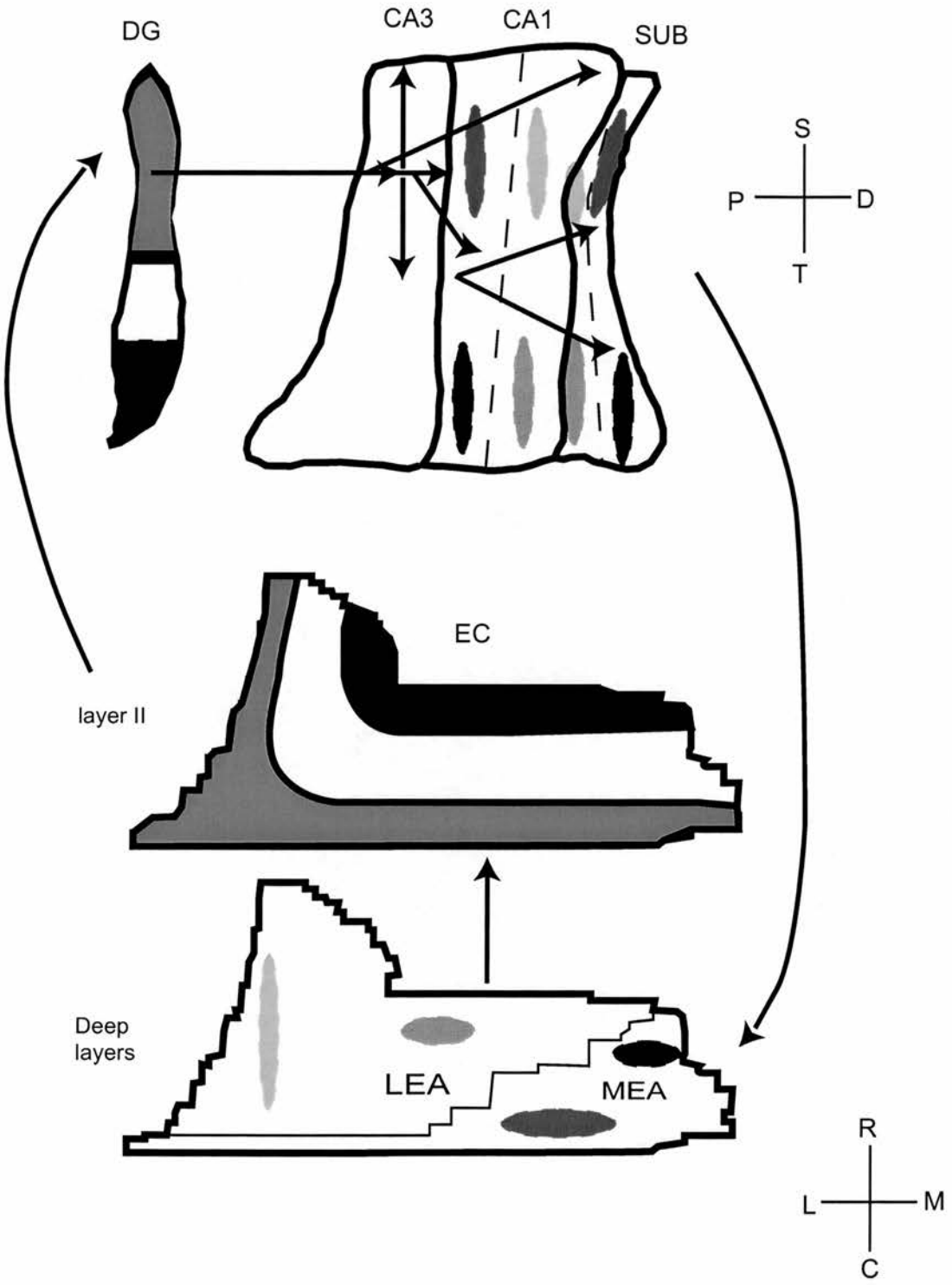


Figure 4.13: The septotemporal segregation of inputs originating in a particular DG-projecting EC band is not necessarily maintained as information travels along the hippocampus intrinsic circuit. Activation of the septal DG can generate activation in proximal CA1 and distal subiculum, which, in turn, activate rostromedial EC, i.e. a band that projects to temporal DG.
S: septal; T: temporal; P: proximal; D: distal; R: rostral; C: caudal;
L: lateral; M: medial.

CA1, specially in proximal regions. This also means that activity originating in one particular band of the EC will be transmitted along the hippocampus and will return to the original EC band but also to adjacent bands.

The same can be said about cortical and subcortical connections with the hippocampal formation, i.e. their original septotemporal segregation will be lost as activity travels through the hippocampal circuit.

This organization allows for separate processing of inputs reaching the hippocampus from different origins and consequent comparisons between these inputs.

4.5 Neurochemical septotemporal gradient

Based on inmunocytochemistry and radioactivity studies in the rat, different neurotransmitters and modulatory substances have been found to vary in concentration along the hippocampal septotemporal axis. This variation is likely to be associated with segregations in connectivity.Inhibition of noradrenaline (NA) synthase or lesions in NA containing nuclei result in 40% decrease of dopamine (a product in the chain of reactions that lead to NA) in dorsal hippocampus (Verney et al. 1985). It is suggested that NA might be synthesised only in the dorsal hippocampus. Dopamine terminals, on the other hand, are mainly concentrated in ventral hippocampus (Verney et al., 1985).

Acetylcholine density in the adult rat is much higher in the temporal half of the hippocampus and is concentrated in specific layers of DG, CA1 and subiculum (Milner et al., 1983). Carbachol (a muscarinic receptor agonist) was used to activate muscarinic receptors (Garcia Ruiz et al., 1993) and it was shown to act more efficiently and with higher affinity in the increase of inositol phosphate in ventral hippocampus. Although there were no septotemporal differences in the concentration of muscarinic receptors, carbachol recognizes three agonist-affinity states in ventral hippocampus and only two in the dorsal part.

Enkephalin is found to terminate only in the ventral parts of CA1 (Gall et al., 1981).

Substance P is a putative neurotransmitter in central and peripheral nervous system. It has been found to be maximally concentrated in the temporal tip of the hippocampus (Mantyh et al. 1984) in CA1, hilus, dentate gyrus and subiculum.

Oxytocin is a posterior pituitary (or neurohypophisis) peptide together with vasopressine. It is known to regulate phenomena like lactation and parturition. It is found in the ventral subiculum (van Leeuwen et al., 1985). Similarly vasopressin-containing fibres are found only in the temporal hippocampus (Caffe et al., 1987).

Thus, temporal levels of the hippocampus are characterized by a higher density of modulators and secondary neurotransmitters. This might lead to a more complex modulation at this level.

4.6 Physiology

Physiological evidence for a septotemporal segregation of hippocampal aspects is not abundant. The strongest suggestion lies in studies that address the susceptibility to epileptic activity in the cat and human hippocampus. A description of the few studies addressing this issue follows but discussion will be undertaken in the experimental chapters.

Elul (1964b) found that discharges in the ventral, but not the dorsal, hippocampus of the cat occurred at a frequency similar to that of epileptic seizures in humans. Surprisingly, recordings from epileptic men (Brazier, 1970), revealed that responses evoked by certain kind of information such as visual stimulation (flash) are higher in the posterior (septal in rodents) CA and DG. Bragdon et al. (1986) found that the ventral hippocampus was more prone to epileptic-like seizures in rat hippocampal slices exposed to potasium. Similarly, Schaffer collateral stimulation in CA1 in a rat slice resulted in higher complex spike responses under normal and high concentrations of potasium in ventral slices compared to dorsal slices (Gilbert et al., 1985). As mentioned before, Li and collaborators (1994) showed that all the labelled cells in temporal CA3 displayed a feedback projection while only some septal ones did. Although based on very few cells, they argued that this effect could be relevant

to the regional sensitivity to epilepsy in humans, where the feedback would create an over activation of granule cells that might overpower the negative feed back carried by basket cells. This effect would be stronger in temporal hippocampus.

Perforant path transmission is fast in fibres that innervate the septal dentate gyrus and decreases as fibres innervate more temporal levels (van Groen and Lopes da Silva, 1985).

The hippocampus is very vulnerable to ischemic processes. Ashton et al. (1989) found that in rats submitted to eight minutes of ischemia (occlusion of carotid and hypothension) electrophysiological activity in the CA1 region had decreased by 70% in the dorsal hippocampus and only by 10% in the ventral part. Histological studies revealed that 90% of the cells were affected by ischemic coagulative cell change at septal levels and only 10% in the temporal area.

4.7 Behaviour

Few studies have addressed the behavioural implications of the septotemporal segregation. Here I describe those studies undertaken prior to Moser et al. (1993). More modern studies are discussed in the experimental chapters, as they form the basis for the design of selected experiments.

In 1965, Gross and colleagues found no difference between the effects of dorsal or ventral lesions to the rat hippocampus in a spatial alternation task. However, the lesions were electrolytic and extended beyond the boundaries of the hippocampus.

Also in 1965, Hughes found that complex maze learning was disrupted by dorsal but not ventral lesions. A result that was replicated later by Sinnamon et al. (1978).

Performance on a fixed interval reinforcement schedule (a DRL task), was also differentially impaired by dorsal and ventral hippocampal lesions. While rats with dorsal lesions behaved like controls (Ellen et al., 1964; Haddad et al., 1967), rats

with ventral lesions showed a higher rate of responding (Haddad and Rabe, 1967; Grant and Jarrard, 1968).

Douglas (1967) argued that the difference between the septal and temporal poles of the hippocampus resides on the degree of involvement rather than on its nature and that many of the differences found are the result of lesions of different sizes. When looking at the histology in many of the papers referenced here, one does find that the lesions were less than well constricted to the hippocampus and that no volumetric measurements had been taken. This makes the interpretation more difficult. Also some of these studies were based in electrolytic lesions which are known to disrupt fibres of passage and, therefore, might well damage circuits beyond the boundaries of the lesion.

Nadel (1968) also revealed a differential effect of electrolytic lesions to the dorsal and ventral rat hippocampus. Activity in a novel situation, habituation to contexts varying in familiarity, acquisition of a conditioned emotional response (CER) and one-way active avoidance response were studied. While both rats with dorsal and ventral hippocampal lesions increased their activity in novel situations compared with control, habituation to a novel environment is only affected in the ventral lesioned rats. In the CER task, the capacity to withhold the instrumental response (drinking water) after CS-US pairing was affected in both groups. While dorsal lesioned rats displayed an increased response latency, this latency decreased in ventral lesioned rats (this result was not replicated in cats where both types of lesions resulted in a decrease in latency, or inability to suppress the response, Andy et al., 1967). In the one-way active avoidance response the opposite result was found. While dorsal lesioned rats decreased their latency to respond, ventral lesioned rats increased it. Nadel attributed a modulatory function of motivational responses to the dorsal hippocampus and associated the ventral hippocampus with behavioural flexibility. Apparently, he gave up with this idea soon after.

In 1968, Siegel and Flynn performed an experiment in which cats with lesions to the dorsal or the ventral hippocampus were tested for their readiness to attack rats. Attack responses were induced by hypothalamic stimulation. Combined stimulation

of ventral hippocampus and hypothalamus resulted in a decrease in attack latency. Combined stimulation of dorsal hippocampus and hypothalamus resulted in an increase in latency. When cats with ventral hippocampal lesions were given hypothalamic stimulation the attacks were suppressed. Dorsal hippocampal lesions, however, generated an inconsistent pattern of results.

Coscina and Lash (1969) did not find support for a functional differentiation, as lesions to neither pole of the structure impaired passive avoidance. Combined lesions, however, did result in deficits in performance in this task.

In 1973, Stevens and Cowey published a series of experiments in which differences, although not necessarily in function, were found between the effects of dorsal and ventral hippocampal lesions in rats. While T-maze spontaneous alternation at long delays was unimpaired in dorsally lesioned rats, ventral lesions spared alternation at short delays. Surprisingly in a lever-alternation task, dorsal lesions rended superior performance compared with controls while ventrally lesioned rats were impaired. Finally, in a simultaneous spatial discrimination were one stimulus is rewarded 70% of the time and the other 30% of the time (differential partial reinforcement schedule), rats with ventral lesions were impaired with respect to both rats with dorsal lesions and controls. Stevens and Cowey concluded that the dorsal hippocampus was involved in attention and habituation and the ventral hippocampus, in modulation of response strategies.

Koreli (1977) designed an experiment that measured the effects of the dorsal and ventral hippocampus on hypothalamic self-stimulation. The effects of concurrent stimulation or ablation of the dorsal or the ventral hippocampus on both acquired responses to positive self-stimulation of the lateral hypothalamus and avoidance responses to electrical stimulation of the medial or ventromedial hypothalamus, was studied. He found that only concurrent stimulation or ablation of the ventral hippocampus had an effect. This effect led to inhibition of positive self-stimulation in the first paradigm and substitution of the avoidance response by a self-stimulating one in the second paradigm. The interpretation was that ventral hippocampus inhibits hypothalamic motivational centres.

The effect of dorsal and ventral lesions in a spatial location order recognition task was measured (Chiba et al., Soc. Neurosc. Abstr., 1992). The task, performed in a radial arm maze, consisted on allowing the rat to enter one arm at a time out of eight in an order established by the experimenter. In the test the rat was presented with two arms from which it had to chose the one that had been presented first in the sequence. The arms could be consecutive (in the time sequence of presentation, not in space) or separated by two, four or six order positions. Dorsal lesioned rats were unable to chose correctly between consecutive arms or those which had two arms between them but were fine at the cases in which there were four or six arms between the arms for choice. Ventral lesioned rats performed as cortex lesioned ones and did fine in all the tests. Surprisingly, in another study (Chiba et al., 1994), rats with complete hippocampal lesions were impaired at all intervals. One would expect the sum of the effect of dorsal and ventral lesions to be equal to the effect of a complete lesion if the dorsal and ventral parts worked independently. According to what has just been described for the anatomy, this is probably not the case, and these results seem to support it.

The results of some these studies suggest a functional differentiation along the hippocampal longitudinal axis, others, however, do not. Even those that do suggest a differentiation do not, when considered as a group, yield an obvious interpretation. For this reason before the study by Moser et al. (1993), no theory had been put forward.

4.8 Conclusions

There are aspects of the connectivity of the hippocampus that are fairly homogeneous along the longitudinal axis, others, however, are not. The intrinsic circuit has a general transverse pattern that is repeated along the whole septotemporal extent of the hippocampus, suggesting a homogeneous processing capacity along this axis. The extrinsic connectivity, on the other hand, is not always homogeneously distributed along the hippocampal septotemporal axis, such that different levels are connected either to different structures or to different regions of the same structure.

This septotemporal variation has functional relevance, as detected in both physiological and behavioural tests, but their nature and significance is unclear. In the following chapters I am going to explore further the implications of this differentiation for hippocampal function.

Chapter 5

Evidence for a functional differentiation along the hippocampal septotemporal axis: replication of Moser et al. (1995)

Table of Contents

Chapter 5

Evidence for a functional differentiation along the hippocampal septotemporal axis: replication of Moser et al. (1995)

5.1 Introduction

In the 1960s, interest arose in the possibility that septal and temporal parts of the hippocampus could differ in function (Elul, 1964a and b; Nadel, 1968; Koreli 1977). Moser et al. (1993) revisited this issue and reported evidence of a functional difference between the two poles of the hippocampus: lesioning the septal, but not the temporal, hippocampus impaired spatial learning.

Moser and colleagues used aspiration lesions which damage fibers as well as cells. A considerable proportion of the extrinsic connections to the whole of the hippocampus course through the dorsal hippocampus. Thus, dorsal aspiration lesions could also de-afferent the ventral hippocampus. For this reason Moser and colleagues replicated the study in Edinburgh in 1995 using ibotenic acid lesions.

Moser et al. (1993 and 1995) trained rats with lesions of different sizes, starting either from the temporal or the septal pole, in a reference memory task in the watermaze. The training consisted of 6 days in which the rats received four consecutive trials in the morning and four in the afternoon. Transfer tests were carried out at the beginning of days 5 and 7. The result (Fig. 5.1) was that rats with 20 to 40% or more of the hippocampus spared from the septal pole, searched as effectively as shams in the final transfer test. Rats with hippocampus spared from the temporal pole required as much as 60 to 80% to reach above chance performance in

the final transfer test. Therefore, while lesioning more than the septal 20 to 40% of the hippocampus impaired spatial memory, lesioning as much as the temporal 60 to 80% of the hippocampus had no effect on performance.

The conclusion was drawn that, while the septal hippocampus is essential for spatial learning, the temporal hippocampus is not and, might, therefore, play a different role such as in non-spatial memory. A functional differentiation along the longitudinal axis of the hippocampus would have important implications for the controversy surrounding its function (described in Chapter 1).

This chapter presents a partial replication of key groups of the 1995 study using the same protocol and apparatus. The reason to replicate this study is that findings presented in Chapter 7, opened questions that Moser et al. did not consider, making it necessary to establish the replicability of their study.

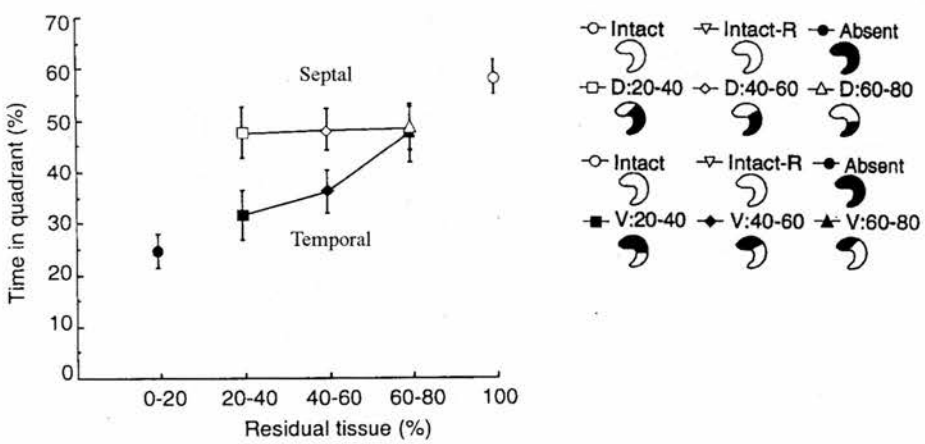


Figure 5.1: Percentage time in training quadrant during final transfer test (day 7) as a function of percentage and location of hippocampus spared. Insert shows residual hippocampal volume as an unfitted icon. D: dorsal hippocampus spared; V: ventral hippocampus spared. From Moser et al., 1995.

5.2 Methods

The experimental protocol was maintained as similar as possible to that used by Moser et al. (1995). However, only a subset of the groups used in their study was

tested here. The apparatus was the same used by Moser et al., whose 1995 study was carried out in Edinburgh.

5.2.1 Ibotenic Acid lesions

Rats were given either sham lesions or ibotenic acid lesions (see Methods, p. 24) aiming to spare approximately the temporal 30% or the septal 30% of the hippocampus.

5.2.2 Behavioural testing

Reference memory task (see Methods, p. 32):

- 6 days of training. 2 blocks of 4 consecutive trials per day. Blocks of trials were separated by at least 4 hours. Total number of trials= 48.

- Transfer tests at the beginning of day 5 (after 32 trials) and on day 7 (after 48 trials).

5.3 Results

5.3.1 Lesions

A total of 55 rats were used of which 1 died after surgery and 45 where accepted after histological analysis. Thus, the following groups were obtained: rats with septal hippocampus spared, averaging 36 ± 1% and ranging between 20 and 44% (n=16); rats with temporal hippocampus spared, averaging 32 ± 2% and ranging between 20 and 41% (n=10); and sham lesioned rats (n=19).

5.3.2 Behaviour

All rats displayed a normal swimming posture and were similar in their capacity to climb onto the platform. Latency to find the platform decreased with training, as illustrated in Figure 5.2.

Acquisition curve

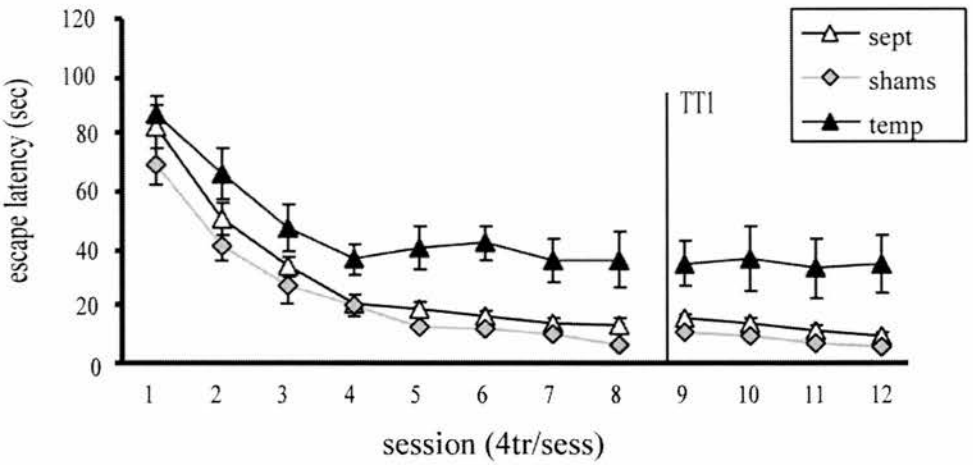


Figure 5.2: Escape latency across sessions (1 session = 4 consecutive trials). Sept and temp: septal and temporal hippocampus spared groups, respectively.

An overall analysis of escape latency across sessions revealed an effect of Group ($F [2, 42] = 21.9$, $p < 0.001$), a decrease in latency across Sessions ($F [11, 462] = 62.6$, $p < 0.001$), but no Group by Session interaction ($F [22, 462] < 1$). Post hoc multiple comparisons (Dunnett 2-sided) revealed that septal spared rats (Mean (M) = 80.9 s on the first 4 trials and 11.4 s on the last 4 trials) were no different from shams (M = 69.6 to 6.3 s) but found the platform faster than temporal spared rats (M = 87.5 to 35.2 s; $p < 0.001$). Temporal spared rats were also significantly slower than shams ($p < 0.001$).

During the first transfer test (Fig. 5.3.a), shams displayed a clear preference for the training quadrant, septal spared rats showed a small bias and rats with temporal hippocampus spared were at chance. An overall analysis of percentage time spent in

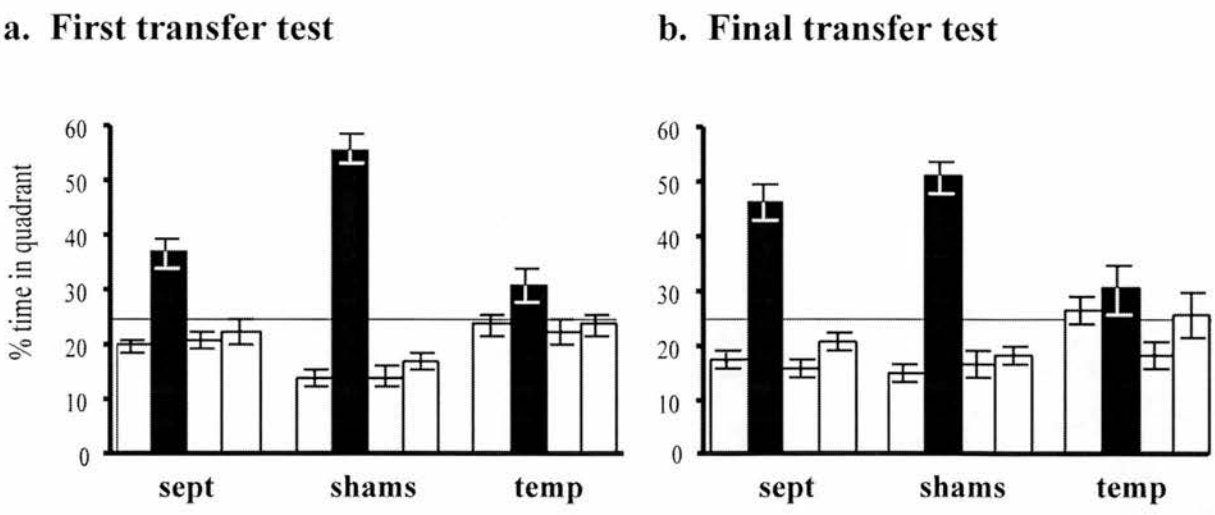


Figure 5.3: Percentage time in each quadrant during the first **(a)** and final **(b)** transfer tests (performed at the beginning of day 5, after 32 trials; and on day 7, after 48 trials, respectively). Sept and temp: septal and temporal spared groups, respectively. For each group the black bar represents the training quadrant; the two adjacent bars, represent the adjacent quadrants; and the forth bar represents the opposite quadrant. Horizontal 25% line represents chance performance.

each quadrant of the pool revealed an effect of Quadrant ($F [1.9, 78.1] = 52.3$, $p < 0.001$; degrees of freedom corrected for sphericity as explained in Methods, p. 35) and a Group by Quadrant interaction ($F [3.7, 78.1] = 12.4$, $p < 0.001$). Analysis of % time in training quadrant (TQ) only, revealed a difference between Groups ($F [2, 42] = 19.2$, $p < 0.001$). Multiple post hoc comparisons (Newman-Keuls) revealed that septal (M = 36.7% TQ) and temporal (M = 30.8%) hippocampus spared rats did not differ from each other, but both searched in a less focused way than shams (M = 55.6%; ps < 0.01).

During the final transfer test (Fig. 5.3.b) the septal spared group clearly differentiated from temporal spared rats and searched in a fashion similar to shams. An overall analysis of percentage time in quadrant revealed an effect of Quadrant ($F [2.2, 93] = 44.7$, $p < 0.001$) and a Group by Quadrant interaction ($F [4.4, 93] = 5.3$, $p < 0.001$). Analysis of the training quadrant only revealed a difference between Groups ($F [2, 42] = 8.3$, $p < 0.001$). Multiple post hoc comparisons (Newman-Keuls) revealed that temporal spared rats (M = 30.3% TQ) spent less time in the TQ than both septal

spared ($p < 0.05$) and shams ($p < 0.01$). Septal spared (M = 46.06%) and shams (M = 50.71%) did not differ (see Fig. 5.4).

8795- 29% septal

4357 sham

4326- 24% temporal

Figure 5.4: Representative swim paths taken by individual rats during the final transfer test. %: percentage tissue spared.

These result constitute a successful replication of the Moser et al., (1995) study. In Figure 5.5 data are represented in the same fashion as in Moser et al. (1995) and included here as Figure 5.1.

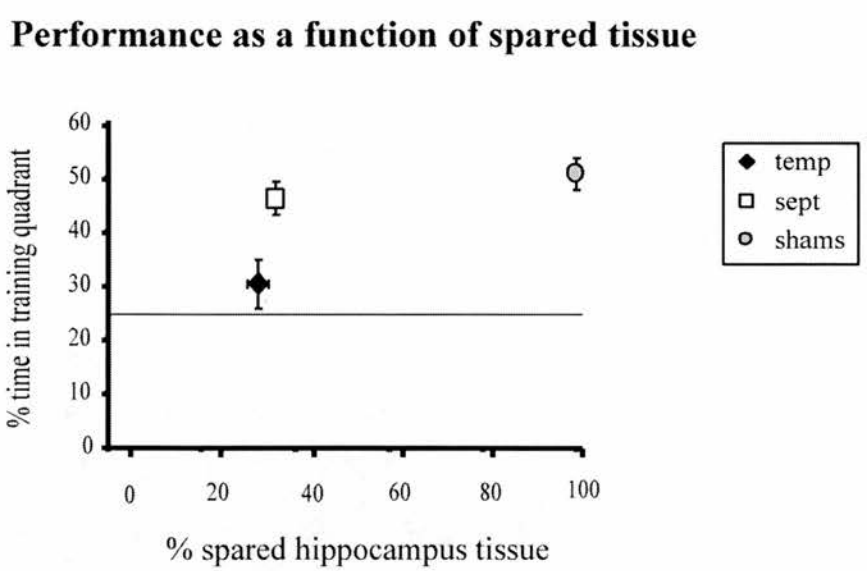


Figure 5.5: Percentage time in training quadrant as a function of amount and location of hippocampal tissue spared. Compare with Figure 5.1 taken from Moser et al. (1995).

Although the results from the final transfer test reflect a clear difference between septal and temporal hippocampus spared rats, it is still possible that the latter group has some residual spatial memory that is neither persistent nor accurate enough to be revealed with the type of measurement used. For this reason, other aspects of the transfer test were analyzed.

For example, it is possible that rats with temporal hippocampus spared swim in the area where the platform was positioned but that, not finding it there, soon extinguish their spatial bias. This might be revealed by analyzing the first 30 seconds of the transfer test.

An analysis of the percentage time in each quadrant during the first 30 seconds of final transfer test (Fig. 5.6.a) exposed an effect of Quadrant ($F [2.2, 94.5] = 38.7$, $p < 0.001$; corrected for sphericity) and a Group by Quadrant interaction ($F [4.5, 94.5] = 5.9$, $p < 0.001$). Analysis of training quadrant only revealed a difference between Groups ($F [2, 44] = 9.6$, $p < 0.001$). Multiple post hoc comparisons (Newman-Keuls) revealed that rats with septal hippocampus spared (M= 43.96% TQ) and shams (M = 54.1%) did not differ from each other but spent more time in the training quadrant ($p < 0.05$) than rats with temporal hippocampus spared (M = 28.5%).

Another possibility is that, although rats with temporal hippocampus spared spend equal time in all quadrants, their search is more localized when swimming over the training quadrant. This should be revealed by analyzing the percentage time in an area of 20 cm in radius around the platform position and comparing it with the percentage time in equivalent areas (centered around what would have been the platform position) in the other three quadrants.

An analysis of percentage time in area (see Fig. 5.6.b), exposed no difference between Groups ($F [2, 42] = 1.8$, $p > 0.2$), but an effect of Quadrant ($F [1.8, 74.2] = 46.6$, $p < 0.001$) and a Group by Quadrant interaction ($F [3.5, 74.2] = 7.3$, $p < 0.001$). An analysis of training quadrant only revealed a difference between Groups ($F [2, 44] = 8.8$, $p < 0.001$). Post hoc comparisons revealed that rats with septal hippocampus spared (M = 14.6% TQ) and shams (M = 18.7%) did not differ from

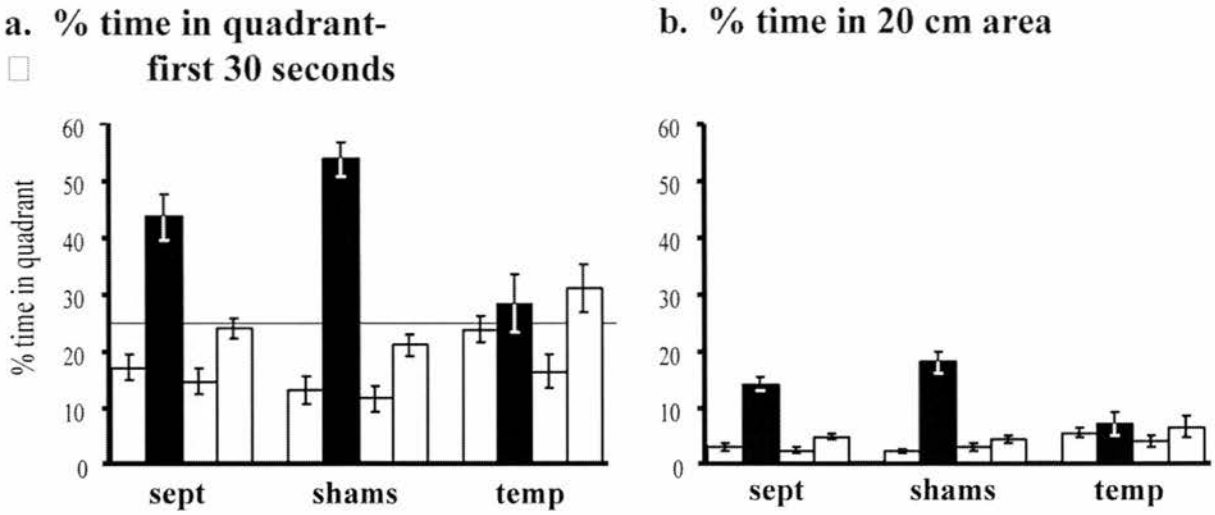


Figure 5.6: Percentage time spent in each quadrant during the first 30 seconds of the transfer test **(a)** and percentage time spent in an area (20 cm in radius) around the platform position or equivalent in each quadrant during the final transfer test **(b)**. Quadrant colour and position coded as before.

each other but both searched more time in the area ($p < 0.05$ and $p < 0.000$, respectively) than rats with temporal hippocampus spared (M = 7.3%).

Do rats with temporal hippocampus spared 'know' where the platform is, even though they do not spend more time in the quadrant or the area where this is located? This might be exposed by analyzing the number of annulus (platform position) crossings with respect to crossing over equivalent positions in other quadrants.

An overall analysis of annulus crossings (Fig 5.7.a) revealed no effect of Group ($F [2, 42] < 1$), an effect of Quadrant ($F [1.9, 81.9] = 21.3$, $p < 0.001$) but no Group by Quadrant interaction ($F [3.9, 81.9] < 1$). Septal and temporal spared groups showed mean annulus crossings in the training quadrant of 2.2 ± 0.3 and 2.0 ± 0.6, respectively. Sham mean was 2.7 ± 0.5. Two things become apparent in Figure 5.7.a: first, that rats with temporal hippocampus spared had a slight bias towards the correct quadrant in terms of annulus crossings and, second, that all the groups displayed higher crossings in the opposite quadrant than in the other non-rewarded quadrants. This effect appeared as a result of the rats being placed in the opposite quadrant at

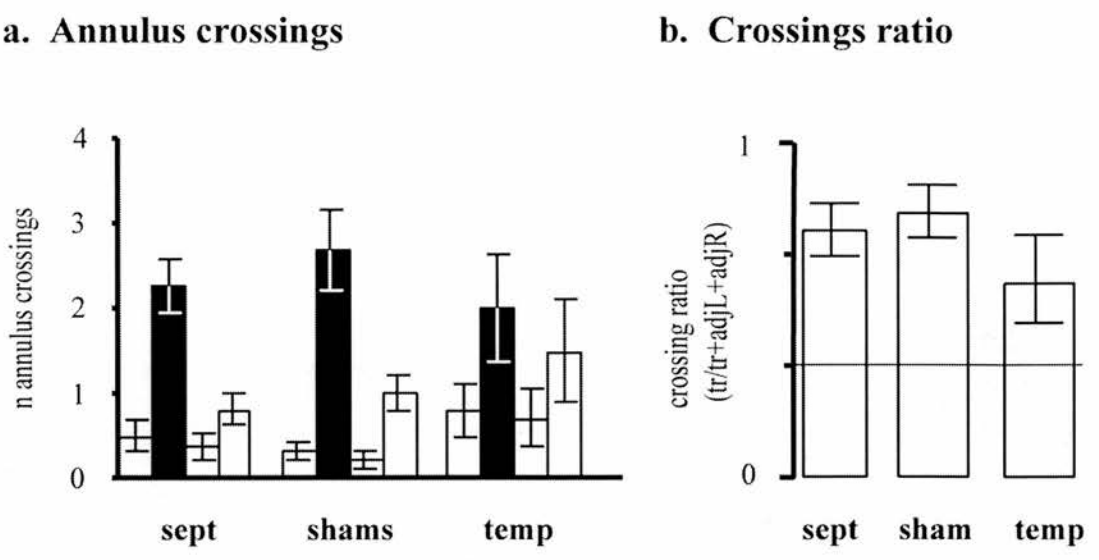


Figure 5.7: Number of annulus crossings **(a)** in each quadrant (colour and position coded as before) and ratio of crossings **(b)** over training quadrant (with respect to all the quadrants excluding opposite). Sept and temp: septal and temporal hippocampus spared groups.

the beginning of the transfer test and of their tendency to swim towards the middle of the pool. For this reason I felt justified to exclude the opposite quadrant from future aspects of the analysis. Consequently, an analysis of crossing ratio (Fig 5.7.b), measured as platform crossings in the training quadrant relative to crossings in all the quadrants (with the exception of the opposite), revealed that temporal spared rats (M = 0.60 ± 0.13) were only slightly worse than septal spared (M = 0.75 ± 0.07) and sham (M = 0.79 ± 0.07) rats. Temporal spared rats, however, were not significantly above chance level (t = 2.1, p = 0.07), indicating that the higher number of annulus crossings over the training quadrant, was a trend resulting from only some of the animals displaying this behaviour.

Running the watermaze, it is obvious to the experimenter that rats slow down their swimming speed as they acquire the task across training sessions. An overall analysis of the average swimming speed during the final transfer test revealed a difference between Groups ($F [2, 42] = 6.7$, $p < 0.01$). Multiple post hoc comparisons revealed that both septal (M = 29 ± 1 cm/s) and temporal (M = 30 ± 1 cm/s) spared rats were significantly different from shams (M = 26 ± 1 cm/s; $p < 0.05$ and $p < 0.01$ respectively) but not from each other (Fig 5.8). Thus, rats with septal hippocampus

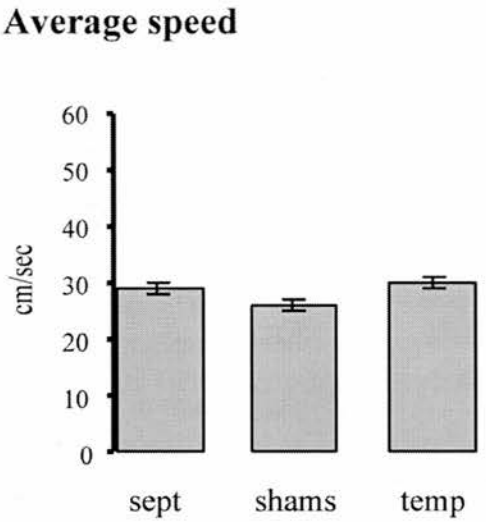


Figure 5.8: Average swim speed during the final transfer test.

spared do not decrease their swimming speed in parallel to them acquiring a spatial bias for the correct location.

How are these alternative measures related to each other and to the traditional measure of percentage time in quadrant over 60 seconds? Percentage time in quadrant during the first 30 seconds and percentage time in 20 cm area during the 60 seconds are measures of persistence and are clearly not independent from the traditional measure. Moreover, the average speed is also a confounded measure of a spatial bias. It is obvious to the experimenter that rats that develop a spatial bias, swim slower. During a transfer test, rats that expect to find the platform in the training quadrant slow down as they swim over this quadrant. Thus, the lower swim speed is not just a general effect, but one that affects the training quadrant as well. The annulus crossings measure, on the other hand, is independent from the others as it reflects accuracy, rather than persistence. It is not surprising then, that it is this latter measure that unveils a residual spatial capacity in the temporal group, that was not revealed by the other measures.

5.4 Discussion

5.4.1 Septal, but not temporal, hippocampal lesions impair spatial learning

The key findings are that lesions to the septal but not the temporal hippocampus impair spatial memory in the conditions tested. The results are identical to those found by Moser et al. (1993 and 1995). This is made evident in the comparison between graphs 5.1 and 5.5. During the final transfer test, after 6 days and 48 trials of training, rats with the septal 20 to 44% of the hippocampus spared displayed a spatial bias towards the correct quadrant, while rats with the temporal 20 to 41% of the hippocampus spared were at chance. Rats with septal hippocampus spared were indistinguishable from shams in terms of escape latencies during acquisition and, also, in terms of percentage time spent in the training quadrant during the final transfer test. However, a slower rate of learning is suggested by the impairment with respect to shams during the first transfer. Not having a group of rats with smaller volumes of septal hippocampus lesioned, it is impossible to say whether this effect is due to the size of the lesion or to the absence of the temporal hippocampus. Also, during the final transfer test, certain measurements, such as swimming speed, revealed that rats with septal hippocampus spared behaved differently from shams and more like rats with temporal hippocampus spared. Again, it cannot be determined whether this is due to the size of the lesion or its mere presence.

5.4.2 A small amount of hippocampal tissue can support spatial memory

These results suggest that as little as 20 to 44% of the hippocampus, located septally, is enough to support spatial learning of a watermaze reference memory task.

From the temporal spared groups, only rats with the temporal 60 to 80% of the hippocampus spared were also able to learn the task (Moser et al., 1995, see Figure 5.1). But this group had between 10 and 30% of septal hippocampus spared and it is, therefore, possible that their good performance is a result of the septal sparing. This would imply that, within the septal hippocampus, different areas can support spatial

learning, as is suggested by the results of Moser et al. (Soc. Neurosci. Abstr., 1997). This supports the idea of non-topographical mapping by hippocampal place cells (O'Keefe, 1976; Wilson and McNaughton, 1993). It is possible that it also constitutes evidence for 'multiple representations of a given environment', as argued in Moser et al. (1995). However, this would depend on whether the different septal minislabs are encoding for similar or different spatial elements.

The sufficiency of small portions of the hippocampus located at different levels within the septal hippocampus has been interpreted as redundancy within the structure (Moser et al., 1995). It is possible, however, that as the complexity of the task increases, the minimum amount of tissue required to learn the task becomes bigger and the redundancy within the structure, consequently decreases. This question is addressed in Chapter 6.

The minimum amount of hippocampal tissue is located bilaterally. However, rats with unilateral sparing may also be able to learn the task. This, too, is addressed in Chapter 6.

5.4.3 Residual spatial memory in rats with temporal hippocampus spared?

The results observed by Moser et al. (1995) that lesions to the septal, but not the temporal, hippocampus impaired spatial memory were replicated.

However, rats with temporal hippocampus spared displayed a trend towards a spatial bias in terms of ratio of annulus crossings in the training quadrant relative to other quadrants. Moser et al. (1995) do not report this measure.

This result is surprising considering that this group was impaired in terms of time spent in either the correct quadrant (after both 30 and 60 seconds) or the area around the platform position. As they spent equivalent amounts of time in the four different quadrants, this means that, when they swim across the training quadrant they preferentially swim over the platform position. Is this an efficient spatial strategy? It is tempting to believe that when a smaller number of cells is available, the

environment is represented with lower resolution. This could affect the temporal hippocampus differentially, as suggested by evidence that, in normal rats, ventral hippocampus place fields are bigger than dorsal fields (Jung et al., 1994; although no difference in place field size is found by Poucet et al., 1994). Thus, temporal spared rats might develop place fields that cover a big area of the pool, but whose peak firing maintains a certain amount of spatial selectivity, allowing the rat to swim over the platform position once in the training quadrant. The swim path of a temporal hippocampus spared rat could then be interpreted as a magnification of a sham's swim path. Thus, with a bigger pool, this path might have appeared more localized in space, at least in relative terms. Zhang and Sejnowski (1999), however, argue that size does not matter, at least when coding two-dimensional information, and that bigger place fields do not result in less resolution, unless accompanied by a decrease in the number of cells.

The effect that partial lesions to the hippocampus might have on place cells is not known. It would be interesting to repeat the experiment presented here while recording units from both septal and temporal hippocampal tissue.

Another, but related, view of the high annulus crossings observed in the temporal group would be that rats in this group did not know where to swim but were capable of recognising a relevant spatial position when they happen to swim there. That rats with hippocampal lesions are capable of recognizing locations of behavioural significance has been previously suggested by Whishaw and Jarrard (1996).

5.4.4 The functional differentiation hypothesis

Lesions to the septal, but not the temporal, hippocampus impair spatial learning suggesting that there is a differential involvement of both poles of the hippocampus in spatial memory. Moser and Moser (1998b) propose that hippocampal functions might be segregated along the septotemporal axis such that the septal hippocampus is responsible for spatial memory while the temporal hippocampus is involved in other, non-spatial types of hippocampal dependent memory.

As discussed in Chapter 1, theories of hippocampal function are controversial but can, roughly, be divided into 'spatial' (O'Keefe and Nadel, 1978) and 'not-exclusively spatial' (Olton et al., 1979; Rawlins, 1985; Eichenbaum et al., 1992; Rudy and Sutherland, 1995). A functional differentiation within the hippocampus, especially one that proposes a dichotomy between spatial and non-spatial theories, would represent an invaluable step forward towards the understanding of the role of the hippocampus in memory.

In the next two sections, anatomical and behavioural findings in support of the functional differentiation hypothesis are presented. However, a more thorough discussion is reserved for Chapter 7, where these, and other studies that do not support the aforementioned hypothesis, are discussed with respect to the results presented in that chapter.

5.4.5 Anatomical support for the functional differentiation hypothesis

There are numerous examples of extrinsic hippocampal connections that support the idea of a functional differentiation along the hippocampal longitudinal axis. As described in Chapter 4, entorhinal cortex DG-projecting bands are organized such that visual and olfactory sensory information from perirhinal and postrhinal cortices reach, mainly, the septal hippocampus. The temporal hippocampus on the other hand, is differentially connected with the amygdala, by means of reciprocal projections, and projects to the hypothalamus. This pattern suggests, that the septal hippocampus processes exteroceptive sensory information while the temporal hippocampus deals with information of emotional relevance. As spatial processing is highly dependent on sensory information, the anatomical data have been understood (Moser and Moser, 1998b) to support the hypothesis that the septal hippocampus is differentially involved in spatial learning. The temporal hippocampus, on the other hand, might be involved in non-spatial, maybe emotional, types of memory. This idea is further discussed in Chapter 7, together with other aspects of the anatomy of the hippocampus that, because of their homogeneity along the septotemporal axis, do not support the functional differentiation hypothesis.

5.4.6 Behavioural support for the functional differentiation hypothesis

In Chapter 4 a series of experiments were described that pointed towards a differential effect of septal and temporal hippocampal lesions on behaviour. The results obtained in these studies do not easily match the spatial versus non-spatial dichotomy suggested by Moser and Moser (1998b). In fact, Nadel (1968), suggests that the dorsal hippocampus has a role in motivational aspects of memory, a function that, according to the functional differentiation hypothesis would more readily be attributed to the ventral hippocampus.

However, evidence in support of a differential involvement of the septal hippocampus in spatial memory is found in other studies published after 1994.

Studies addressing the question of whether there is functional differentiation along the septotemporal axis of the hippocampus are scarce. Hock and Bunsey (1998) found that rats with septal hippocampus spared were better (in terms of errors to criterion) than rats with temporal hippocampus spared in the delayed version of a T maze alternation task (considered to be an example of a spatial task). Temporal spared (dorsal lesion) rats, however, did reach criterion eventually (the number of trials to criterion per group is not stated). Also as, in this study, dorsal lesions were bigger than ventral lesions, the difference in time to reach criterion could be related to the amount of tissue spared rather than its location. Bannerman et al. (1999) found that septal, but not temporal, lesions to the hippocampus impaired watermaze performance (trained over 6 days at a rate of 4 trials per day) and T maze alternation, thus supporting the functional differentiation notion as introduced by Moser and Moser (1998b). An accompanying study, however, found that dorsal hippocampal lesions did not result in impairment in a watermaze trained with an identical protocol (Richmond et al., 1999). Vann et al. (2000) found that c-fos activation was greater in the dorsal hippocampus of rats trained in a spatial radial arm task but only when they were tested in a novel environment. Activation of c-fos was increased, but equivalent in dorsal and ventral levels of the hippocampus, in rats trained and tested in a single radial arm maze. Thus, these results support a differential involvement of the septal hippocampus in spatial memory only under certain conditions. Olsen et al. (1994)

found that four vessel occlusion (known to affect cells in the septal hippocampus more readily than cells in the temporal hippocampus) affected spatial learning and reversal in a watermaze. There was a correlation (though small due to rats with very few cells spared performing well) between the number of viable cells and escape distance. Finally, Mao and Robinson (1998) found that muscimol injection into the dorsal, but not the ventral, hippocampus impaired some aspects of an operant spatial delayed-matching to position. However, working memory and delayed choice accuracy was intact in both conditions.

In a different line of work, Blum et al. (1999) found that MAP-Kinase activation in hippocampus of rats trained in the watermaze was limited to the septal part of the structure.

The differential involvement of the septal hippocampus in spatial memory extends to humans. Maguire et al. (2000) compared the size of the hippocampus of taxi drivers (high spatial exposure) with that of controls along the anteroposterior axis. They found that posterior levels (septal in rats) of the hippocampus of taxi drivers were larger than those of controls. Moreover, the size of the posterior hippocampus in taxi drivers was positively correlated with the time these subjects had spent in their profession.

Although behavioural studies addressing the possibility of a functional differentiation within the hippocampus in monkeys do not exist, unit recordings point in a similar direction. Colombo et al. (1994, 1998) found that cells in the posterior part of the monkey hippocampus (equivalent to septal in the rat) were more responsive to spatial components of the task, while temporal hippocampus cells respond preferentially to egocentric, directional aspects. This is, however, a quantitative, not qualitative, difference as cells responding to all the given parameters were found along the whole extent of the hippocampus.

Because of the easier accessibility of the dorsal hippocampus, numerous studies, although not directly addressing the question of a functional differentiation, support the findings that the dorsal hippocampus is involved in spatial learning (Phillips and LeDoux, 1994; Riekkinen and Riekkinen, 1997). These results, however, do not

necessarily support the notion of a functional differentiation, as the effect of equivalent manipulations of the ventral hippocampus were not studied.

What might be the function of the temporal hippocampus? The roles attributed to the temporal hippocampus are in direct connection with the specific projections to and from this part of the hippocampus. Thus, the projection from the ventral hippocampus directly and via the lateral septum, to specific hypothalamic nuclei (Risold et al., 1997) have led to the idea that the ventral hippocampus is differentially involved in the control of autonomic and neuroendocrine responses. The ventral subiculum is also likely to exert an important control over these kind of functions (Herman and Cullinan, 1997), through projections to the bed nucleus of stria terminalis (Cullinan et al., 1993). Evidence for this role is found in Henke (1990) where lesions to the temporal hippocampus, but not the septal, result in aggravation of gastric erosion following restrain stress. Lesions to the ventral subiculum also cause glucocorticoid hypersecretion following restrain stress and exposure to open field (Herman et al., 1998). Tuvnes et al. (Soc. Neurosci. Abstr., 1999) found no difference in fear-driven freezing response between septal and temporal hippocampus lesioned rats. They did, however, find increase in plasma glucocorticoid in the latter group.

The temporal hippocampus is known to influence the hypothalamic-pituitary-adrenocortical axis (HPA) in relation to stress responses. For example, ventral levels of the hippocampus inhibit this axis in response to psychological but not systemic stressors (Nettles et al., 2000). Decrease in plasma arginine vasopressin (pAVP) is found after activation of the ventral hippocampus in response to psychological stress. Lesions to this part of the structure block this effect, a result that has led to associate the ventral hippocampus with some neuropsychiatric disorders. For example, a subset of individuals with schizophrenia characterized by episodes of life-threatening water intoxication, show increased levels of pAVP (an antidiuretic hormone whose level decreases after water consumption) and a reduction in volume in the anterior (temporal in rats) hippocampus (Luchins et al., 1997).

Also, it is suggested that the hippocampus mediates the modulation of the HPA axis by the degree of maternal care during the first 10 days of life in rats (Liu et al., 1997). More licking and grooming by the mother in early life results in an increase in glucocorticoid receptors in the hippocampus and reduced corticosterone releasing hormone in the periventricular nucleus of the adult offspring. Although this study did not address this question directly, this effect is likely to be mediated by the temporal, rather than the septal, hippocampus.

Temporal levels of the hippocampus have also been associated with modulation of locomotor activity. Lesions to the temporal hippocampus increase both spontaneous and amphetamine induced locomotor activity in the rat (Lipska et al., 1992). These changes in activity are associated with changes in dopamine turnover in the nucleus accumbens. Accordingly, dopamine turnover in the basal ganglia is affected by lesions to the temporal hippocampus (Lipska et al., 1992, 1993) but not by lesions to the septal hippocampus (Lipska et al., 1991). Whether modulation of locomotor activity and related dopamine changes in the basal ganglia can be considered as a hippocampal role rather than a secondary influence is difficult to say.

The implication of the functional differentiation hypothesis, as it stands, is that it should be possible to find a double dissociation, i.e. a non-spatial task dependent on the temporal, but not the septal, hippocampus. Such a task has not been found to date. Studies attempting to find a double dissociation are discussed in Chapter 7, together with findings that do not support the differential involvement of the septal hippocampus in spatial memory.

5.4.7 Conclusions and further questions

The result obtained by Moser et al. (1995) are replicated. The septal, but not the temporal, hippocampus is necessary for rats to learn a reference memory task in the watermaze, trained over 6 days at a rate of 8 trials per day.

As little as 20 to 40% of the structure is sufficient to learn this task.

It is not known, however, whether the minimum amount of tissue is the total amount of hippocampus spared (around 30% of the total bilateral hippocampus) or whether an equivalent but unilateral sparing (30% of unilateral hippocampal volume) would also be sufficient. Similarly, it is not known, whether the minimal amount of tissue varies with the type and demands of the task used. These two questions are addressed in Chapter 6.

Many anatomical and behavioural findings support the notion of a functional differentiation along the septotemporal axis of the hippocampus as proposed by Moser et al. (1998b). The longitudinal extent of intrinsic associational projections and some behavioural findings, however, suggest that differences along the septotemporal axis might not result in a clear functional differentiation, but rather in a longitudinal variation on a unitary hippocampal function. This hypothesis is tested in Chapter 7 and the literature discussed thoroughly with respect to the results obtained.

Chapter 6

Aspects of the organization of the residual septal hippocampus and their contribution to different forms of spatial memory

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Chapter 6

Aspects of the organization of the residual septal hippocampus and their contribution to different forms of spatial memory.

6.1 Introduction

Two questions are addressed in this chapter. The first question refers to the basic organization of the circuit required to solve the reference memory task, specifically whether commissural connections (interhemispheric connections within a structure) across equivalent septotemporal levels of the hippocampus contribute to spatial memory in a similar manner to associational connections along the septotemporal axis. If one understands the bilateral 30% group (in the previous chapter, 20-40%) as formed by two slabs of tissue, one on each hemisphere, the question is whether spatial learning is a result of the capacity of each of those slabs or of the joined action of both slabs. Would one of those slabs (unilateral 30% or 30+0) be as efficient as the bilateral version (30+30)? Or would a quantity of tissue equivalent to the sum of the two slabs (unilateral 60% or 60+0) be needed? If the latter, are the commissures between the bilateral 30% slabs as efficient as associational projections within the unilateral 60%? Answering these questions will help us understand the basic limitations and needs of the circuit that supports spatial learning.

The second question refers to the issue of whether the volume of hippocampus sufficient to learn a simple reference memory task also has the processing capacity to use the spatial information in a more flexible and demanding manner. This is addressed by training the groups described above in a delayed-matching to place (DMP) task, after completion of the reference memory task. In this task the platform

position is changed everyday and the animal is given four consecutive trials per day. Latency to reach the new platform position in trial 2 is used as a measure of one-trial learning. Thus, the rat needs to navigate attaching different values to different positions on different days.

In summary, this chapter explores (i) whether spatial learning can be achieved with unilateral hippocampus only; if so, (ii) is the minimal unilateral amount of tissue necessary to learn a reference memory task in the watermaze equivalent to one of the slabs of the bilateral minimal amount or to the sum of both? (iii) how increasing the demands of the task affects the efficiency with which the above described circuits support learning.

What follows is a brief overview of the anatomical organization and behavioural consequences of hippocampal commissures. Associational connections within the hippocampus with special reference to their septotemporal extent have been thoroughly reviewed in chapter 4 and further discussed in chapter 5. They will, therefore, be omitted in this introduction.

6.1.1 Anatomy

There is a general rule on the organization of commissural projections that applies also to the hippocampus: not all associational connections have a commissural equivalent but all commissural projections have an equivalent associational connection.

The two hippocampi are connected profusely (Blackstad, 1956) though differently in different species: e.g.; the commissural systems of the rat and monkey are not alike (Amaral et al., 1984). The connections of the rat occur through the ventral-hippocampal commissure and are neither exclusively homotopic (Gottlieb and Cowan, 1973; Hjorth-Simonsen and Laurberg, 1977) nor homogeneous along the longitudinal axis: sectioning of the ventral commissure results in more degeneration in the septal hippocampus relative to the temporal hippocampus (Laurberg, 1979). The commissures arise from the hilar region and from CA3 (Gottlieb and Cowan, 1973; Laurberg, 1979; Tamamaki and Nojyo, 1991).

The commissural projections have become a useful model for different types of research because they are easy to manipulate on their way through the fimbria fornix. The system has been used to study the interaction of fiber tracts in the hippocampus (Buzsaki and Czeh, 1981;Buzsaki and Eidelberg, 1981; Douglas et al., 1983; Bilkey and Goddard, 1987), to demonstrate the basic principles of synaptic connectivity such as feed forward inhibition (Frotscher and Zimmer, 1983; Leranth and Frotscher, 1983; Seress and Ribak, 1983, 1984; Buzsaki, 1984; Deller and Leranth, 1990; Deller et al., 1994), and to study synaptic reorganization after entorhinal cortex lesion (Zimmer, 1973; West et al., 1975; Nadler and Cotman, 1978; Gall et al., 1979).

6.1.1.1 Hilar commisural projection to the dentate gyrus

The hilar commissural projection originates from around 80% of the different types of hilar neurons (Ribak et al, 1986) and terminates in the contralateral dentate gyrus in the inner and outer thirds of the molecular layer (Hjorth-Simonsen and Laurberg, 1977; Laurberg and Sorensen, 1981; Swanson et al., 1981; Voneida et al., 1981; Bakst et al., 1986; Deller et al., 1995).

The hilar commissure terminating in the inner third of the molecular layer has a small transverse distribution but a broad septotemporal extension (Gottlieb and Cowan, 1973), with low density at the septotemporal level of origin but high density in levels more septal or temporal to the injection (Deller et al., 1995). Hilar neurons project more densely to the septal contralateral side (Hjorth-Simonsen and Laurberg, 1977).

The commissure terminating in the outer third of the molecular layer, on the other hand, has a broad transverse distribution but a narrow longitudinal extent.

6.1.1.2 CA3 commissural projection

The projection to CA3 and CA1, innervates both contralateral CA3 and CA1, projecting to, at least, the septal ¾ of CA3 including homotopic areas (Gottlieb and

Cowan, 1973; Laurberg, 1979). The projection to the hilus is small and, probably, the result of collaterals from the CA3 to CA3 commissure fibres (Deller et al., 1995).

6.1.1.3 Species differences.

Little is known about the human commissural system in the hippocampus. There is evidence that hippocampal function in humans is lateralized (Milner and Taylor, 1970). The left hippocampus appears to be more item specific than the right hippocampus (Nyberg et al., 1996), with items, like words, being processed in that side. It is also more active than the right hippocampus when tasks require language to either be explained or be solved. This could be due to the lateralization of language more than the actual lateralization of the hippocampus. It has been suggested that the left hippocampus is involved in encoding (Lacquaniti et al., 1997), and that the right hippocampus is involved in retrieval (Maguire et al., 1997). More data are required to substantiate this argument.

The commissural system of the monkey differs from that of the rat. In the hippocampal formation it is restricted to the more rostral part of the structure (uncal region). The uncal region of the monkey hippocampus is the only part that shares similarities with the rat hippocampus and would correspond to the temporal part of the latter (Amaral et al., 1984).

6.1.2 Behavioural work

That spatial learning can be achieved with one side of the hippocampus temporarily inactivated by lidocaine was first demonstrated by Fenton and Bures (1994) and Fenton et al. (1994). In their study though only the dorsal part of the hippocampus in one side was infused, leaving the ventral part of the hippocampus unaffected bilaterally. Lidocaine inactivation is also different in nature to ibotenic acid lesions. The former acts as an anaesthetic and blocks activity in the fibres of passage, while the latter kills the cells in a permanent way with minor effects on the fibres of passage.

Fenton and Bures (1994) found that an animal can learn a reference memory task in the watermaze during temporary inactivation of one hippocampus. If retrieval was performed while the trained side , but not the naïve side, was inactivated the rats were impaired. This suggested that, between trials sessions, when lidocaine had washed out, information was not transferred between the two sides of the hippocampus. In an ingenious follow up, the left hippocampus was inactivated while the rat was presented only with cues A and B in order to locate the platform. Then, the right hippocampus was inactivated and only cues C and D were made available. The rats learnt to find the platform very quickly with each side of the hippocampi active separately. Testing took place while both hippocampi were active and with various cue combinations. Rats found the platform as quickly using the training cues AB or CD as using the alternative combination of these, i.e. AD or CB. These data suggest that information that is stored separately in both hippocampi can be retrieved together and combined, but that this cross-talk between the hippocampi is context and/or retrieval dependent.

Bannerman and colleagues (personal communication) have evidence that unilateral NMDA lesion to the hippocampus has no effect on a T maze task. This task is very different from a watermaze task and it is not possible to infer from it the effect that unilateral damage might have on spatial learning.

In this chapter, the question is addressed of whether bilateral minislabs of septal hippocampus act as two independent units or as a unique circuit.

6.2 Methods

6.2.1 Ibotenic acid lesions

Rats underwent surgery and partial lesions were made (see Methods, p. 24) aiming to spare the septal 30% of the hippocampus bilaterally (30% bilateral group), the septal 30% of the hippocampus unilaterally (30% unilateral group) or the septal 60% unilaterally (60% unilateral group). The percentage refers to what is spared if 100% is one side of the hippocampus. A subset of the animals received sham lesions. Some

of the animals included in the bilateral and sham groups presented in the previous chapter, are also included here as the training protocol is identical (up to the end of the reference memory task).

6.2.2 Behavioural testing.

Rats were left to recover from surgery for at least 10 days before training started.

Reference memory task (see Methods, p.32): as in the previous chapter

- 6 days of training, with 2 blocks of 4 consecutive trials per day. Total number of trials = 48.

- Transfer tests at the beginning of day 5 (after 32 trials) and on day 7 (after 48 trials).

Delayed-matching to place (DMP, see Methods, p. 33):

- intertrial intervals (ITI) between trial 1 and 2 varied between 5 seconds, 20 minutes and 2 hours for the different days.

- ITI between trials 2 and 3, and trials 3 and 4, was maintained constant at 5 seconds.

- Starting positions and platform positions were counterbalanced such that they were equally distributed across groups, ITIs and days.

- 5 days pretraining with the different ITIs.

- 9 days of training. Each rat had 3 days of each ITI.

6.3 Results

6.3.1 Lesions

A total of 50 rats received either partial or sham ibotenic acid lesions of the hippocampus. Three animals died after surgery and, from the 47 remaining, 30 were accepted after histological analysis.

The final numbers were as follows. The 30% bilateral group was formed of 8 rats, whose spared tissue averaged 37 ± 2%, and ranged between 28 and 42%. A subset of these animals was included in the septal 30% group presented in Chapter 5. The 30% unilateral group was made of 7 rats, averaging 34 ± 1 % spared tissue and ranging between 29 and 39%. The 60% unilateral group was formed of 5 rats, with an average of 54 ± 3 % spared tissue and ranging between 45 and 65% spared. The sham group was formed by 10 subjects, a subset of which was included in the study presented in Chapter 5.

6.3.2 Reference memory task

All rats swam with the normal adult swimming posture and were able to climb onto the platform.

Acquisition escape latencies were found to decrease across sessions (Fig. 6.1). An overall analysis revealed an effect of Group ($F [3, 26] = 4.9$, $p < 0.01$) and a decrease on escape latency across Sessions ($F [11, 286] = 74.2$, $p < 0.001$), but no Group by Session interaction ($F [26, 286] < 1$). Post-hoc multiple comparisons between groups (Dunnett 2-sided) revealed that both the 30% unilateral and bilateral groups escaped more slowly than shams ($p < 0.005$ and 0.05, respectively), while the 60% unilateral group did not differ.

An overall analysis of percentage time spent in each quadrant during the first transfer test (Fig. 6.2.a), revealed an effect of Quadrant ($F [2.2, 56.1] = 50.7$, $p < 0.001$,

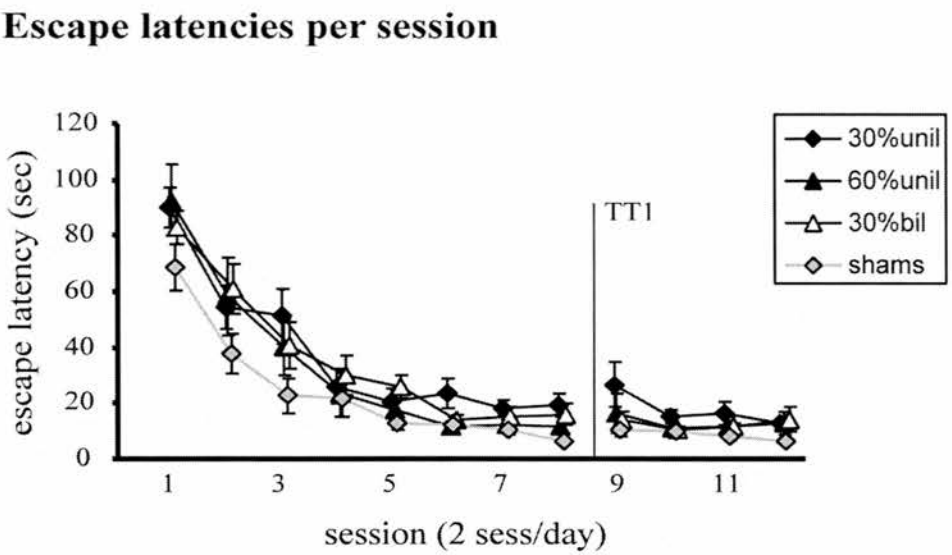

Figure 6.1: Escape latencies per session (2 sessions per day) during acquisition. A first transfer test (TT1) was given at the beginning of day 5 as indicated by the vertical bar. Unil: unilateral hippocampal sparing; bil: bilateral hippocampal sparing.

degrees of freedom corrected for sphericity; see Methods, p.35), and a Group by Quadrant interaction ($F [6.5, 56.1] = 9.3$, $p < 0.001$). An analysis of training quadrant only indicated that there was an effect of Group ($F [3, 29] = 17.6$, $p < 0.001$). Post-hoc multiple comparisons between groups (Dunnett 2-sided) revealed that shams searched in a more focussed manner than each of the lesioned groups ($p < 0.001$ in all cases) and that the lesioned groups did not differ from each other. Although the latter is true of the training quadrant only, Figure 6.2.a makes it apparent that the bilateral group displayed a clearer bias for the training quadrant than the other two groups.

By the final transfer test all groups had acquired a clear and equivalent bias for the training quadrant (Fig. 6.2.b). Accordingly, an overall analysis revealed an effect of Quadrant ($F [1.7, 44.2] = 34.1$, $p < 0.001$) but no Group by Quadrant interaction ($F [5.1, 44.2] < 1$).

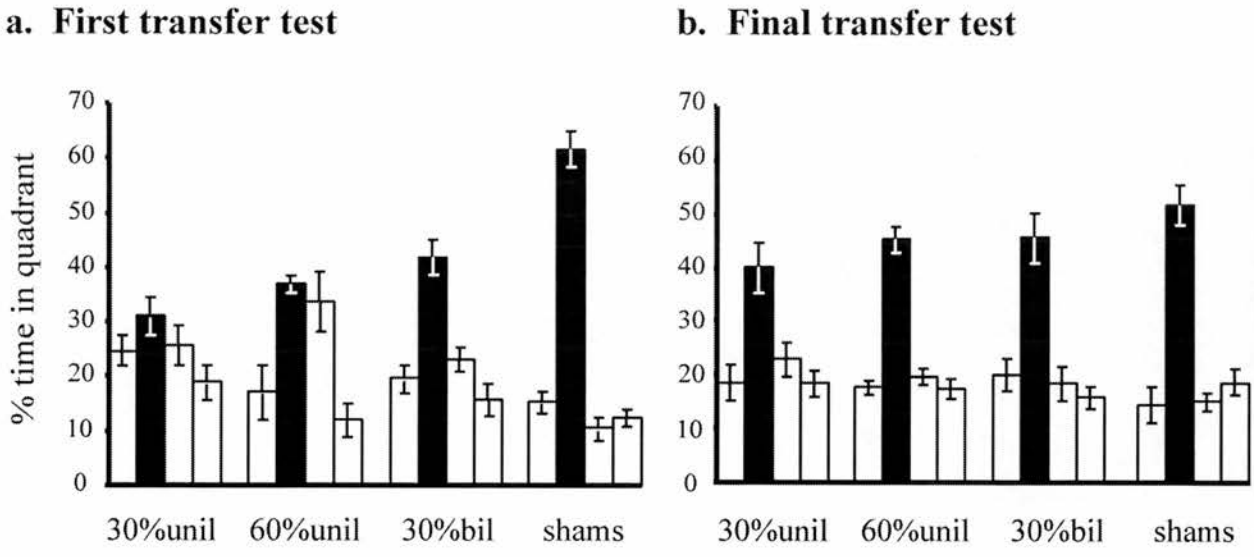

Figure 6.2: Percentage time in each of the quadrants of the pool during first **(a)** and second **(b)** transfer tests. For each group the black bar represents the training quadrant, the left and right adjacent bars represent the adjacent left and adjacent right quadrants respectively, and the far right bar represents the opposite to training quadrant.

6.3.3 Delayed-matching to place (DMP) task

During the first days of training, performance was clearly influenced by the confounded effect of learning a new task and extinguishing another. For this reason, the first 5 days were considered as pre-training and only the data from the following 9 days of training were analyzed in detail. The decision to chose 5 days was based on evidence from Steele and Morris (1999) that rats require approximately 5 days of training before they start doing one-trial learning.

Figure 6.3 illustrates well the general pattern of the task. High latencies for trial 1 indicate that the rat has no previous knowledge of the day's platform position. This latency is specially high in shams and may reflect, in part, sham's memory for the

previous day's platform position. Its search in that location was often observed on trial 1 of the day, and this search delayed its finding of the new platform position. Decrease in latency across daily trials is also observed. This suggests that the rats are reinforced within a day and that, even if some groups do not show one-trial learning, there is no reason to believe their motivation is being extinguished across training.

An overall analysis of escape latency across trials and ITIs (data shown across graphs a, b and c in Fig. 6.3, where the lesioned groups have been averaged into one) revealed an effect of Group ($F [3, 24] = 3.1$, $p < 0.05$), an effect of ITI ($F [2, 48] = 3.5$, $p < 0.05$) and an effect of Trial ($F [3, 72] = 40.2$, $p < 0.001$). Interactions were found across Group by Trial ($F [9, 72] = 5.4$, $p < 0.001$) and ITI by Trial ($F [6, 144] = 2.9$, $p < 0.05$) but not across Group by ITI ($F [6, 48] = 1.7$, $p > 0.05$). No triple interaction ($F [18, 144] = 1.3$, $p > 0.05$) was found.

With the data as they stand, it is difficult, if not impossible, to find a triple interaction. In fact, it is precisely the design of 4 trials per day (discussed in Chapter 2) that, by allowing all groups to acquire a spatial bias and display a decrease in latency across trials at all ITIs, results in a lack of interaction.

However, the presence of an effect of Group and a Group by Trial interaction justifies the analysis of trial 2, as a measure of one-trial learning. The ITI by Trial interaction justifies a separate analysis of each ITI.

A one way ANOVA analysis of trial 2 only in the 5 second ITI condition revealed and effect of Group ($F [3, 27] = 4.5$, $p < 0.05$). Post-hoc multiple comparisons (Dunnett 2-sided) revealed that only the 30% unilateral group was significantly different from shams ($p < 0.005$). Neither the 60% unilateral nor the 30% bilateral groups were significantly different from shams.

No effect of Group was found during an analysis of trial 2 at the 20 minutes ITI condition ($F [3, 27] = 2.5$, $p > 0.05$) or at the 2 hours ITI condition ($F [3, 27] = 1.2$, $p > 0.05$).

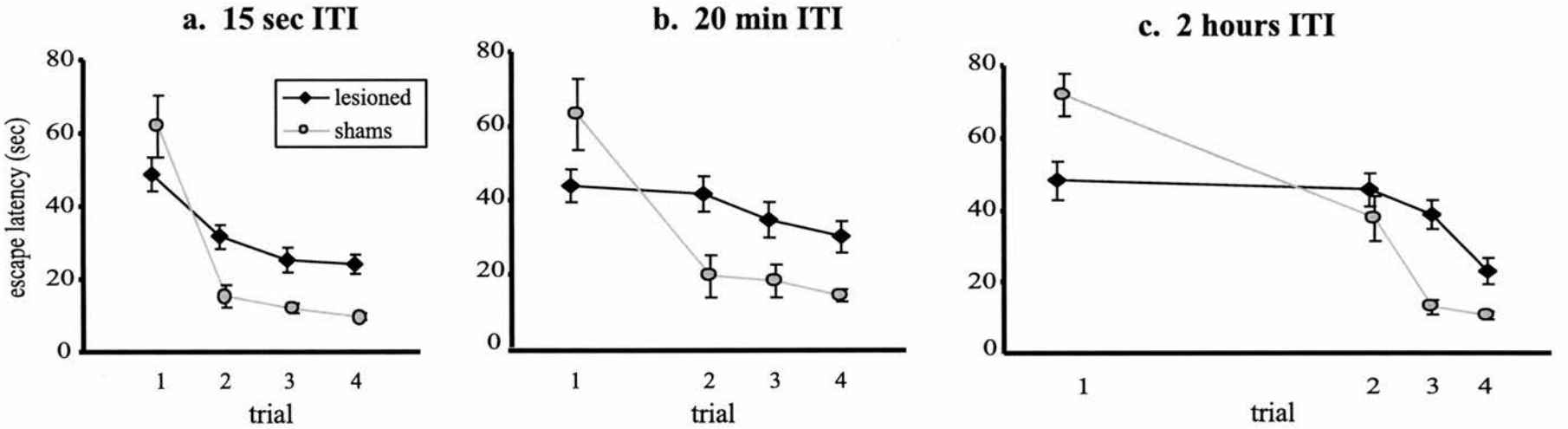

Figure 6.3: Graph illustrating the logic of the DMP task. Average escape latency across trials for the 5 sec **(a)**, 20 min **(b)**, and 2 hours **(c)** ITI. The three lesioned groups are averaged together.

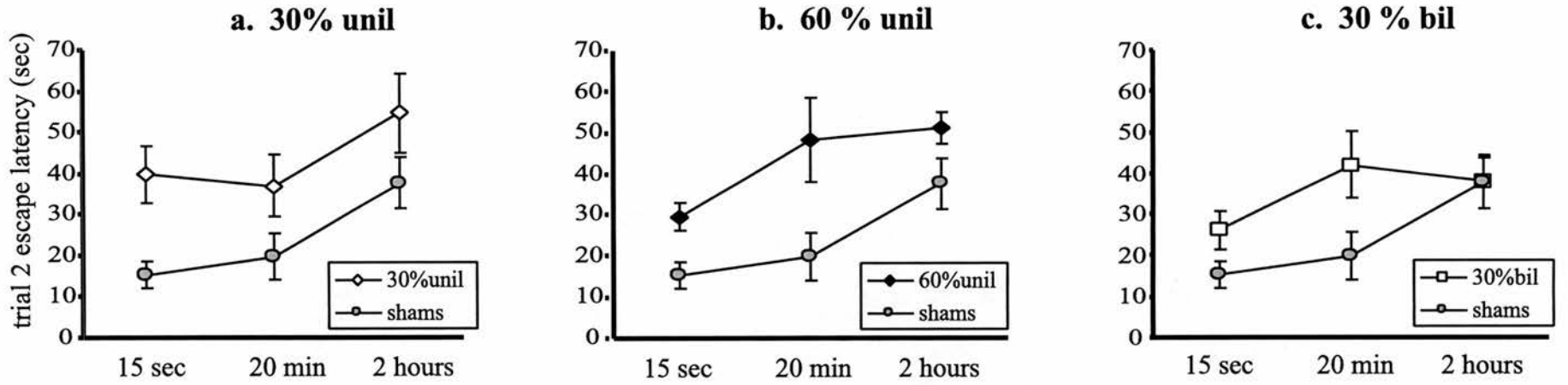

Figure 6.4: Trial 2 escape latency across teh differnt ITIs. Each lesioned group: 30% unilateral **(a)**, 60% unilateral **(b)**, and 30% bilateral **(c)** hippocampus spared, is plotted against the sham lesioned group.

In Figure 6.4, escape latency on trial 2 across ITI is represented for each lesion group against sham. These graphs illustrate sham's forgetting over time of information acquired on trial 1. They also reflect the capacity for 5 second long memory in the60% unilateral and the 30% bilateral groups. It also becomes clear that it is the enormous variability in the lesioned groups after 20 minutes and the forgetting effect in shams after 2 hours that explains why no effect of Group was found at these time-intervals.

Escape latency in trial 2 is a useful measure but not necessarily the best assessment of memory. A look to Figure 6.3 c illustrates this point. Latency in trial 2 for the lesioned groups does not differ from latency on trial 1, in which the animal had no knowledge of the platform position. Therefore, trial 2 latencies cannot, in this case, be used as a measure of memory. One can, instead, measure the savings between trial 1 and trial 2, i.e. how much shorter trial 2 escape latency is with respect to trial 1. The saving are calculated by deducting individual trial 2 escape latencies from the group average trial 1 escape latency (Fig. 6.5).

An overall analysis of savings across the 5 second ITI condition revealed an effect of Group ($F [3, 27] = 6.9$, $p < 0.005$). Post-hoc multiple comparisons revealed that all lesioned groups displayed less savings than shams ($p < 0.05$ for the 60% unilateral and 30% bilateral groups and $p < 0.005$ for the 30% unilateral group). The difference between the result obtained by analysing the savings and that analysing trial 2 escape latency (where only the 30% unilateral group differs from shams) is due to the measure of memory for the previous day confounded in the savings value. A look at Figure 6.5.a, however, reveals positive savings in all the groups indicating that at this time-interval, trial 2 escape latency is a real measure of memory and that the results obtained upon its analysis reflect a real group difference.

A look at Figures 6.5.b and c on the other hand, reveals no savings in the lesioned groups, suggesting that trial 2 escape latency at the 20 minutes and 2 hours ITIs conditions does not reflect memory.

Thus, it can be concluded that all groups display a decline in escape latency across trials and that one-trial memory in the lesioned groups is only observed at the 5 second ITI.

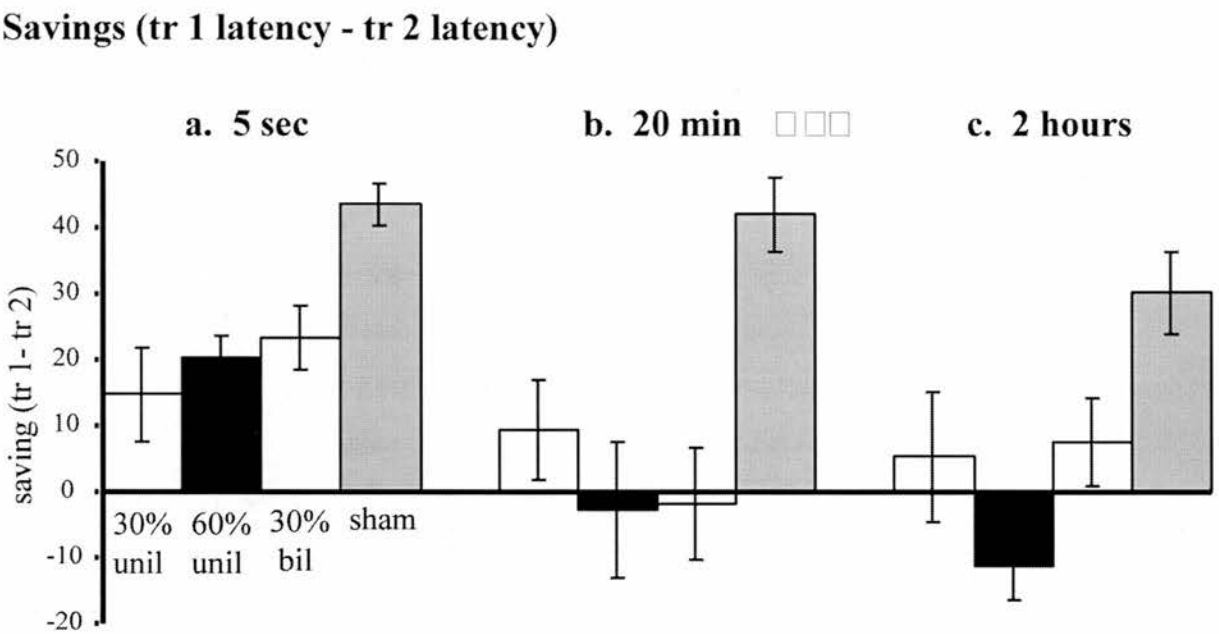

Fig 6.5: Savings between trials 1 (group average) and 2 (individual latency) across groups for each inter-trial interval (ITI): 5 seconds **(a)**, 20 minutes **(b)** and 2 hours **(c)**. Unil and bil: unilateral and bilateral hippocampus sparing, respectively.

6.4 Discussion

In the reference memory task all lesioned groups performed as well as shams during a final transfer test (day 7). In the delayed-matching to place (DMP) task, however, all groups are impaired with respect to shams in terms of savings, although positive savings were seen at the 5 sec ITI, with the 60% unilateral and 30% bilateral groups reaching trial 2 escape latencies comparable to those of shams.

Considering that both tasks where carried out in an identical context and had identical procedural demands (swimming away from the walls, climbing onto the platform, remaining on the platform for 30 seconds), it is interesting, to find such a striking difference in lesioned animals performance between the reference memory and the delayed-matching to place task.

6.4.1 Reference memory task: little and unilateral is enough

In the reference memory task, rats with only unilateral hippocampus spared were as good as shams, even when only 30% of the tissue was spared. This result confirms and extends findings reported by Fenton et al. (1995) and Bannerman et al. (personal communication). The results by Moser et al. (1993 and 1995) and the ones presented in chapter 5 indicate that the minimal amount of tissue necessary to solve the reference memory watermaze task is 30% of the total hippocampus. Here the unilateral equivalent (30% of one side or 15 % of the total hippocampal tissue) is also found to support learning of this task. This group also confirms that the integrity of commissural projections is not necessary to solve this task.

The reference memory task is often used as a sensitive assay of hippocampal function. The finding that even rats with as little as 15% of the total hippocampus spared (30% unilateral group) can learn this task, suggests that the task might not be sensitive enough to disclose the effect that a localized intervention (e.g. drug injection) might have on hippocampal function.

The use of rats with little hippocampus spared might, for this very reason, become a useful tool. In these rats, a localized drug injection or an electrophysiological effect are more likely to affect all the hippocampus spared. Thus, the result observed would reflect the effect of the drug on hippocampal function, rather than the localized effect confounded with the noise attached with having some parts of the hippocampus unaffected by the drug.

Rats with 30% bilateral hippocampus and those with 60% unilateral hippocampus spared displayed equivalent performance during the final transfer test. During the first transfer test, however, the bilateral group showed a greater bias towards the training quadrant. This could be interpreted in two ways. First, one could argue that, as the bilateral's group average spared tissue was 37% (equivalent to 37% of the total hippocampus), whereas the unilateral's average was 54% (equivalent to 27% of the total hippocampus), it is the bigger volume in the bilateral group that is responsible for the trend. It is unlikely, however, that this difference in volume is crucial. A

reanalysis of the bilateral group, excluding rats with more than 35% tissue spared (mean of included rats = 31%), gave a similar percentage time in quadrant measure in either transfer test. Additionally, the temporal 'edge' of the spared tissue is likely to be functionally affected by the disruption of the circuit at that point. The bilaterally lesioned brains have two such edges, while the unilaterals have only one. Although the extent of this dysfunctional edge is not known, it is fair to assume the bilaterals, having two such edges, will be more affected by it. Thus, the initial difference between these two groups is better explained as a facilitation conferred by the presence of commissural connections.

As discussed in the introduction, because of the segregation of extrinsic projections along the septotemporal axis of the hippocampus, a slab located in the septal tip of the hippocampus is qualitatively different from a slab located more temporally. This difference is manifested not necessarily in the processing capacity of the slab but in the type of information processed as this is determined by the origin of the projections received by that slab. If this is so, one would expect that having more temporally located tissue (unilaterals 60%) would give an advantage in this task given that it results in a more heterogeneous connectivity. For example the unilateral and bilateral 30% groups lack the projection to the retrosplenial cortex and projections from the nucleus reuniens of the thalamus that are limited to the mid-levels of the hippocampus. These connections are present in the unilateral 60% group. It is possible, however, that these projections do not contribute in a big way to the reference memory task, whereas having two copies of the same tissue (bilateral 30%) might increase the processing capacity of the circuit. This might explain the initial facilitation conferred by the commissural projections to the bilateral group.

In order to test whether it is the presence of commissures that confers this initial advantage to the bilateral 30% group one would need to explore the effect that a transection of the commissures would have upon the performance of this group.

6.4.2 DMP task: delay-dependent impairment

All lesioned rats displayed some one-trial learning at the 5 seconds ITI, but not at longer ITIs, suggesting that the small amount of hippocampus spared allows for a certain amount of short term spatial memory. Despite this memory, only the groups with 30% of the total hippocampus spared, whether unilaterally or bilaterally, reached trial 2 escape latencies in any way comparable to sham's.

This finding suggests that, at least within the septal hippocampus, it is the total amount of hippocampus spared rather than septotemporal extent that determines hippocampal memory capacity as measured by the DMP task. Thus, adding more temporally located tissue, which according to the anatomy would hold a more assorted selection of connections, does not necessarily add any advantage to the processing capacity of the spared hippocampus in the DMP task.

We know that the commissural projections are organized in such a way that they do not affect the septotemporal gradient. This is due to the general rule that all commissural projections have a homotypic (in terms of cell and level of origin and cell and level of termination) associational equivalent while there is no commissural equivalent to every associational projection. In other words, whether an associational projection has a transverse or longitudinal topography, its commissural equivalent will show an identical topography and, therefore, will not upset the normal septotemporal gradient along the hippocampus. Thus, it would be interesting to study the performance of rats with the septal 30% spared in one side and the midseptal 30% spared in the other side and compare it with the unilateral 60%. This, and other mixed designs, would give us a direct comparison between associational projections and commissural projections between the septal tip and the mid levels of the hippocampus.

6.4.3 Why 5 seconds but not 20 minutes?

Steele and Morris (1999) presented evidence that the memory after the shortest ITI (15 second in their study) in a DMP task, although hippocampal dependent, did not require the activation of the NMDA receptors. Moreover, saturation of LTP does not impair performance in a DMP task when the second trial occurs 15 seconds after the

first, but has very negative effects on performance when the ITI is increased to 20 minutes or 2 hours (Otnaess et al., 1999). As suggested in these studies, short term spatial memory requires the hippocampus but not activation of NMDA receptors. To generate a longer-lasting memory, however, NMDA receptor activation is necessary.

A possible explanation is that the maintenance of memory across a short interval is the result of hippocampus processing of on-line (still active) cortical traces. This on line processing might not require much hippocampal tissue and need not engage NMDA-dependent plasticity. However, when the animal is placed back in the water after 20 minutes or 2 hours have passed, the hippocampus is required to retrieve selectively the memory traces of the last trial but not the previous day's trials, and trigger an appropriate action. In order for these traces to be retrieved they need to have been maintained across an interval of, at least, 20 minutes, for which NMDA receptor activation is apparently required. The complexity of the mechanism by which specific memory traces are rapidly selected and processed might require more volume of hippocampus than that spared in the groups trained in this study.

This hypothesis explains the delay-dependent deficit observed in the lesioned groups, while keeping in line with the findings by Steele and Morris (1999) and Otnaess et al. (1999) described above. The idea that during, at least, short time-intervals memory traces exist outside the hippocampus is supported by findings of a delay-dependent involvement of the hippocampus in working-memory tasks (Bunsey and Eichenbaum, 1995). However, in order to fit the hypothesis above, retrieval of spatial memory must require the integrity of the hippocampus at all delays. There is evidence that this is the case (Aggleton et al., 1986; Jarrard, 1993; Steele and Morris, 1999).

The patient HM is known to be able to maintain memories for up to 20 seconds. It is also known that parts of his posterior (septal) hippocampus are spared (Milner et al., 1968). It is possible that the 30% bilateral and 60% unilateral groups in this study have HM's capacity for the same reasons.

A question that remains outstanding after this study is how much hippocampus is required in order to display normal memory across the 20 minutes and the 2 hours intervals.

6.4.4 Other delayed-matching or non-matching tasks.

Delayed-matching to sample (DMS) and non-matching to sample (DNMS) tasks are widely used for both rats and monkeys. There is controversy about whether these tasks are hippocampal dependent or not and this dependency seems to be related to the type of DMS tasks used and the training protocol (Hampson et al., 1999b). Cassaday and Rawlins (1995) and Rawlins et al. (1993) used a different version of a DMS task and found that the bigger, the simpler and the fewer the stimuli, the strongest is the impairment after hippocampal lesions. When pseudo-trial-unique complex stimuli or small and complex goal boxes are used, hippocampal lesions result in no impairment. Although hippocampal dependency on delayed-matching to sample tasks is controversial, there is strong evidence that, when the task is made spatial, the hippocampus is required at all delays (Aggleton et al., 1986; Steele and Morris, 1999; Hampson et al., 1999b).

DMS tasks are generally synonymous with recognition memory tasks. The DMP task in the watermaze, however, cannot easily be compared with recognition memory tasks. It is not sufficient for the rat to recognize the place where the platform is located today, it also has to navigate to it.

The controversy surrounding delayed-matching tasks and whether they are working and/or recognition memory tasks illustrates the complexity of the mental processes underlying any kind of memory. Thus, although some tasks might seem to require identical processes, it is necessary to be cautious before generalizing across them.

6.4.5 Impairment in the DMP task but not in the reference memory task.

It is interesting that all lesioned rats, unimpaired in the reference memory, are impaired in the DMP task as both tasks are given in exactly the same environment. In fact the only physical difference between the 2 tasks is the position of the platform

(fixed in one task, variable in the other), which the rats cannot see. Sham rats can automatically update information in that environment. This was observed on the first day of pretraining in the DMP task (data not shown). On the first trial, they swam to the position used during the reference memory task and only found the new position by chance or by being led to it when the trial finished at the end of the 120 seconds. On the second trial, they could already pursue a fairly direct path towards the new platform position.

The lesioned rats have demonstrated in the reference memory task that they can navigate through a particular environment and reach a particular position within it. It can be argued that they have created a 'map' of that environment. But it seems that the more flexible use of that 'map' demanded by the DMP task, in which positions acquire or loose their reward component, requires more hippocampal tissue than required to find one unique and constant point in that 'map'. This would fit in with the view that the hippocampus has cells that respond to generic behavioural aspects of tasks (Wood et al. 1999; Hampson et al., 1999a).

Another interpretation could be that with very little hippocampus spared the rats encode information for only the most relevant cues. While the reference memory task requires attention to few relevant cues to be solved (only 2 according to Fenton et al., 1995), the DMP is likely to require a different set of relevant cues for different platform positions. The rapid learning of these would then determine the need for more spared hippocampal volume. However, both the 30% bilateral and 60% unilateral groups can perform the DMP task when the ITI is 5 seconds. Good performance across even the shortest ITI has been shown to be hippocampal dependent by Steele and Morris (1999). The fact that these two lesioned groups can maintain information for that period of time suggests that the tissue necessary to encode that information is there, what is lacking is the machinery to make that information last. This again seems to support the idea that hippocampal tissue encodes information about space (constant during both tasks) and about the flexible use of it and that these two components of the map are not necessarily supported by the same cells or even the same circuits. Consistent with this idea, Foster et al. (2000) present a model of the hippocampus that, although capable of solving a

reference memory task-equivalent, requires the addition of components to solve a DMP task-equivalent.

These arguments, however, do not explain how rats that cannot keep information for 20 minutes can learn the reference memory task, for even in this task the memory has to be maintained from day to day in order to display a decrease in escape latency and a bias towards the correct quadrant during the transfer test. It is possible that the answer resides in the capacity of the lesioned groups to display a decrease in escape latency across the four trials of each day in the DMP task. The close repetition of trials seems to bypass the limitations created by the small volume of hippocampus. This repetition would be even more facilitatory during the reference memory task, where interference between days is non-existent. Another argument could be that the process underlying memory for a constant position in space (a generic situation) is different from that underlying memory for the position that was rewarded in a specific situation (the first trial of the day). This could be understood to support the suggestion that the DMP task tests episodic-like memory in rats. I am reluctant, however, to interpret the DMP task as an episodic-like memory task. For the task to reflect episodic memory, one should be able to conclude that the rat's performance was the result of 'automatic recording of events', using the definition of episodic memory used by Morris and Frey (1997). Here the rat is taught specifically to remember a particular set of parameters decided by the experimenter and, thus, this memory cannot be said to result of an automatic process.

6.4.6 Technical implications.

The interest of the results presented in this chapter resides also in their technical applications. For example, it has been suggested that long-term potentiation (LTP, Bliss and Lömo, 1973) might be the mechanism underlying learning and memory processes. This is a subject of enormous debate (see Martin et al., 2000 for a recent review). One possible approach to test this hypothesis is to explore the effect of saturating LTP on learning and memory. The rationale behind this is that if LTP acts as a memory trace by which synapses that have been previously activated are

differentially marked, inducing LTP in all the synapses should impair both a previous memory and new learning.

However, saturating LTP in the whole of the hippocampus is not an easy task. Saturating LTP in a smaller volume of tissue would be plausible. The fact that the unilateral 30% group can perform the reference memory task converts this group into a useful tool. One could use rats with only the septal and unilateral 30% of the hippocampus spared and saturate LTP in that small block of tissue. In fact, the experimental protocol used by Moser et al. (1998) is based on this approach. They train rats with a complete but unilateral lesion to the hippocampus and saturate LTP on the intact side. Rats in which LTP has been successfully saturated (it cannot be induced anymore) are impaired in the watermaze.

6.5 Conclusions

As little as 15% of the total hippocampus is enough to support spatial memory of a reference memory task in the watermaze. This finding suggests that, when studying the effect a localized injection has on hippocampal function, performance in the reference memory task might not be a sensitive enough assessment of this effect.

The difference in performance between the reference memory task and the DMP task, despite sharing the context and procedural requirements, together with some residual capacity to maintain a new spatial platform location for short periods of time (5 seconds), suggest that hippocampal cells do more than encode for space. It is likely that behavioural information attached to the space is also dealt with by the hippocampus.

These results, as with those in Chapter 5, point to the importance of reporting any hippocampal sparing in the human literature, and of complementing information on memory tests with imaging studies. A lot of the variability found between human cases of amnesia could be due to different volumes of hippocampus spared.

Chapter 7

The temporal hippocampus and spatial memory: evidence for a unitary hypothesis of hippocampal function

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Chapter 7

The temporal hippocampus and spatial memory: evidence for a unitary hypothesis of hippocampal function.

7.1 Introduction

The results obtained by Moser et al. (1993, 1995) and replicated in Chapter 5 suggested that the septal, but not the temporal, hippocampus is essential to learn a reference memory task in the watermaze. These results raised the notion that the septal, but not the temporal, hippocampus is involved in spatial memory. This idea is further supported by anatomical and behavioural evidence, all of which led Moser and Moser (1998b) to propose the functional differentiation hypothesis.

The implications of this hypothesis are very relevant for the study of memory and hippocampal function in particular. The controversy surrounding hippocampal function is, as discussed in Chapter 1, enormous. It would prove invaluable to be able to associate different functions to different areas of the hippocampus. This differentiation would also be of relevance for the study of the functional anatomy as it would then be possible to associate particular memory roles to the particular anatomical features that characterize different regions of the hippocampus.

At this point, two approaches can be taken. One can go forward and explore the nature of the temporal hippocampus dependent function, or one can step back and question whether this functional differentiation applies to all spatial tasks and not just the one tested.

Studies adopting the first line of thought have focused on the search for a double dissociation between septal and temporal hippocampus dependent functions. An overview of these studies is given in the discussion.

The second approach, the one adopted in this thesis, is based on the possibility that the hippocampus is responsible for a unitary memory process and that the anatomical segregation along the longitudinal axis, rather than generate different processing units, is a feature of that general processing capacity. Evidence was presented in Chapter 5 of some remaining spatial bias in the number of annulus crossings in the temporal spared rats. Also, the results presented in Chapter 6, suggest that when the task is modified to demand a more flexible use of the spatial information (delayed-matching to place task), rats with septal spared hippocampus were not capable of maintaining the memory for the newly learnt platform positions. Moreover, recordings of place cells in the temporal hippocampus suggest that this part of the structure might be involved in spatial memory (Jung et al., 1994 and Poucet et al., 1994).

For these reasons, rather than exploring the nature of differential function of the temporal hippocampus, it was decided to further test the spatial memory capacities of this part of the structure.

In this chapter a series of experiments attempting to engage the temporal hippocampus in spatial learning are presented. The task was maintained as similar as possible to that of Moser et al. (1995) in order to ensure that any result obtained was easily comparable with the dissociation found in that study, but another level of difficulty was added. Rats were trained in two concurrent watermazes where the animals had, not only, to learn to navigate and learn the location of a submerged platform but also to distinguish between two contexts, the two rooms in which each of the two watermazes was located. This constituted the two concurrent watermazes experiment (2 WM Expt).

As will become apparent further down, it was necessary to perform a one-watermaze control of the 2 WM Expt. This constituted the one-watermaze experiment (1 WM Expt).

7.2 Methods

7.2.1 Ibotenic acid lesions

Lesions (see Methods, p.24) were made aiming to obtain the following groups in both experiments:

Rats with septal 20 to 40 % of the hippocampus spared.

Rats with temporal 20 to 40 % of the hippocampus spared.

Rats with no hippocampus spared: complete lesions.

Sham lesioned rats.

7.2.2 Behavioural testing

7.2.2.1 Two watermazes.

The protocol is very similar to that of Moser et al. (1995). The difference was that each of the daily training sessions was given in a different watermaze. Pilot studies revealed that 6 days of training resulted in very little learning by any of the groups and, therefore, the training was prolonged for a further 2 days.

The final protocol was as follows:

- 8 days of training

- 4 consecutive trials (1 session) per day in each watermaze. One session was given in the morning and the other in the afternoon. The watermaze corresponding to each session was counterbalanced.

- first transfer test at the beginning of each watermaze session on day 5

- final transfer test on both watermazes on day 9

- 4 consecutive trials in a visible platform task

The visible platform task was run in one of the pools for all the rats.

7.2.2.2 One watermaze control.

Equivalent to the one above but with training in only one of the mazes.

- 8 days of training

- 4 trials per day

- first transfer test at the beginning of day 5

- final transfer test on day 9

- 4 consecutive trials in a visible platform task

Half the animals received all their trials and tests in one pool. The other half was trained in the other pool. The visible platform task was run in the same pool for all the rats.

7.3 Two concurrent watermazes: Results

7.3.1 Ibotenic acid lesions

A total of 61 rats received partial, complete or sham lesions of the hippocampus. Two rats died after surgery and 13 were excluded after histological analysis. The remaining 46 rats were divided as follows: Rats with septal 31 to 44 % hippocampus spared (average of 38 ± 2 %; n = 9), rats with temporal 28 to 44 % hippocampus spared (average of 35. ± 2 % spared; n = 12), shams (n = 14) and rats with no hippocampus spared (n = 11).

7.3.2 Behaviour

All rats swam with a normal adult swimming posture and were similar in their ability to climb onto the platform.

No significant difference in either acquisition or transfer test performance was found between the two watermazes, thus the data were averaged across watermazes.

An overall analysis of acquisition escape latencies (Fig 7.1) unveiled a strong effect of Group ($F [3, 42] = 10.6$, $p < 0.01$) and a decrease in latency across Days ($F [7, 294] = 112.1$, $p < 0.01$) but no Group by Day interaction ($F [21, 294] = 0.7$, $p > 0.05$). Post-hoc group comparisons (Dunnett, 2-sided) revealed that shams (Mean escape latency: 59.7 s on the first day to 7.1 s on the last day) and rats with temporal 28-44% hippocampus spared (M: 73.5 s to 11.7 s), but not rats with septal 31-44% hippocampus spared (M: 75.2 s to 12.9 s), escaped faster than rats with no hippocampus spared (M: 88.1 s to 28 s; $p < 0.01$ and $p < 0.05$, but $p > 0.05$, respectively). Both partial lesioned groups were slower than shams ($p < 0.05$ for both).

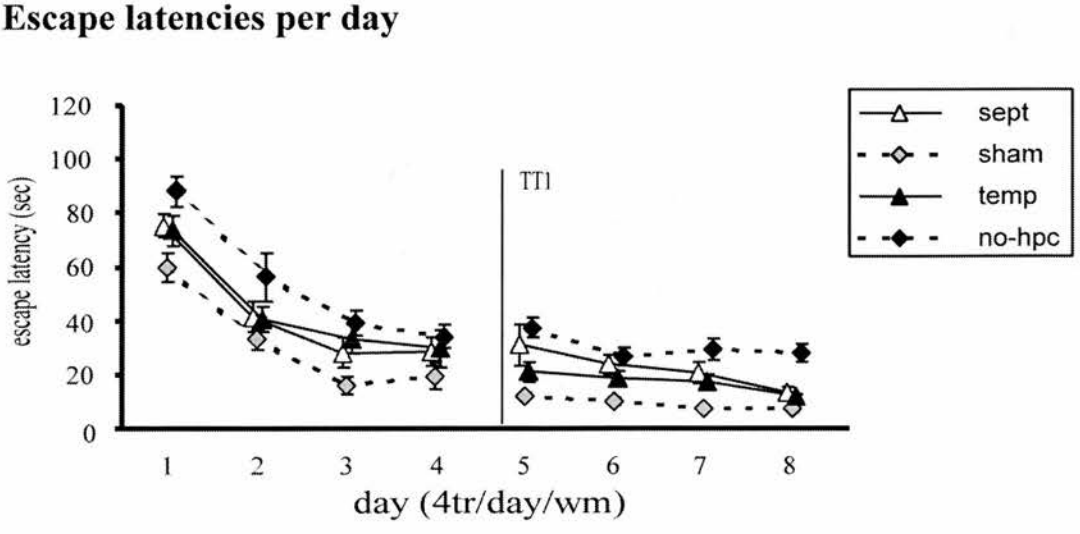

Figure 7.1: Escape latency per day averaged across watermazes. TT1: indicates when the first transfer test was given.

An overall analysis of % time spent in each of the four quadrants during the first transfer test (Fig 7.2.a) revealed a strong effect of Quadrant ($F [2.1, 86.1] = 6.6$, $p < 0.01$, degrees of freedom corrected for sphericity; see Methods, p. 35) and a Group by Quadrant interaction ($F [6.3, 86.1] = 4.5$, $p < 0.01$). Analysis of % time spent in training quadrant only (TQ) revealed a strong effect of Group ($F [3, 42] = 9.9$, $p < 0.01$). Dunnett 2-sided post-hoc comparisons disclosed no significant difference between rats with septal (Mean % time in TQ = 30.4%) and rats with temporal (M= 25.07% TQ) hippocampus spared.

By the final transfer test both septal 31-44% and temporal 28-44% displayed a bias towards the training quadrant. An overall analysis of % time in each of the four quadrants (Fig 7.2.b) revealed an effect of Quadrant ($F [1.9, 78.1] = 66.1$, $p < 0.001$) and a Group by Quadrant interaction ($F [5.6, 78.1] = 4.4$, $p < 0.001$). An analysis of % time spent in the training quadrant only, disclosed a strong effect of Group ($F [3, 42] = 6.4$, $p < 0.01$). Orthogonal post hoc comparisons revealed that septal spared rats (M = 42.9% TQ) did not differ from temporal hippocampus spared rats

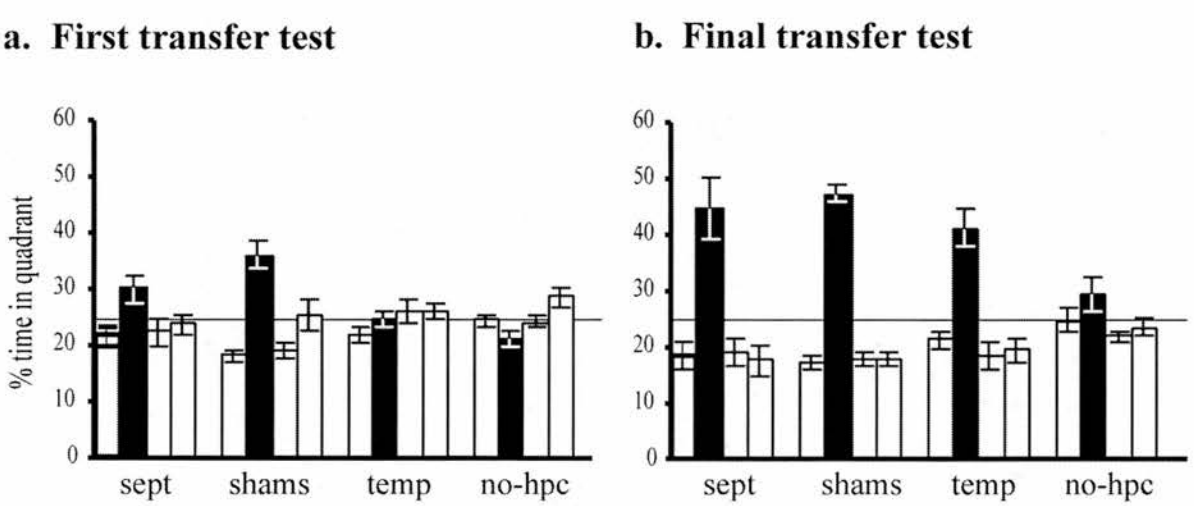

Figure 7.2: First **(a)** and final **(b)** transfer tests. Sept and temp: septal 31 to 44% and temporal 28 to 44% hippocampus spared respectively; no-hpc: no hippocampus spared. For each group: black bar represents the training quadrant, adjacent bars represent adjacent quadrants and far right bar represents the opposite to training quadrant.

(M= 43.7% TQ; F [3, 42] < 1), that both partial lesioned groups were no different from shams (M = 46.2% TQ; F [3, 42] = 1.3, $p > 0.05$) and that shams and partial lesioned rats differed from rats with no hippocampus spared (M = 27.1; F [3, 42] = 16.6, $p < 0.001$).

See figure 7.3 for typical swim paths during the final transfer test.

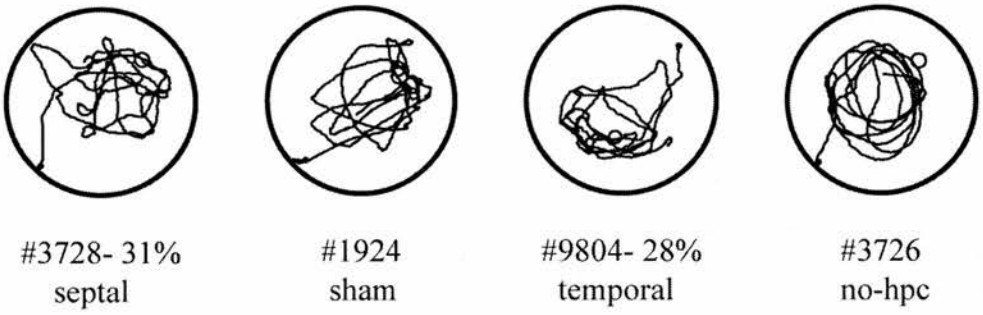

Figure 7.3: Representative swim paths taken by individual rats during the final transfer test. % is % spared tissue.

7.3.3 Visible platform.

All the rats received four trials with a visible platform after the final transfer test. All the animals swam directly towards the visible platform and, by the 4th trial, the escape latency was shorter than 15 sec in all rats. This indicates that none of the rats had a visual impairment which could account for deficits found in the spatial task.

7.4 Interim Discussion

The result obtained in the two concurrent watermazes experiment suggests that rats with less than the temporal 44 % of the hippocampus spared are capable of developing a spatial bias towards the training quadrant in a reference memory task trained in two concurrent watermazes. They are also not different from rats with equivalent amounts of hippocampus spared septally or from sham rats.

Before discussing the implications of this result it is important to note that this experiment was different from that of Moser et al. (1995) in two respects: rats were trained in two, rather than one, watermazes, but they were also given two more days of training. In order to establish the cause of the difference in performance between this study and that of Moser and colleagues, it was decided to perform a one watermaze control of the experiment just described.

7.5 One watermaze: Results

7.5.1 Ibotenic acid lesions

A total of 89 rats received septal, temporal, complete or sham hippocampal lesions. Four rats died after surgery and 15 were excluded after histological analysis. The remaining 66 were grouped as follows. For the main body of the results the following groups were used: rats with septal 24 to 44 % hippocampus spared (averaging 34 ± 2; n = 9), rats with 24 to 41 % hippocampus spared (averaging 33 ± 2; n = 13), rats with no hippocampus spared (n = 9) and sham lesioned rats (n = 17). For analysis of correlations between performance and amount of hippocampus spared the following subjects were added: rats with more than 44 % spared septally (n = 6) or temporally (n = 12) and shams that were trained with the latter but not with the fore mentioned groups (n = 4).

7.5.2 Behaviour

Here the main body of the results is presented and this is based on the septal 24 to 44 % spared and the temporal 24 to 41% spared groups.

An overall analysis of escape latencies per day (Fig 7.4) revealed a strong effect of Group ($F [3, 44] = 7.7$, $p < 0.01$), a decrease in latency across Day ($F[7, 308]=76.2$, $p<0.01$) but no Group by Day interaction ($F [21, 308] = 0.6$, $p > 0.05$). Multiple comparisons revealed that there was no difference between septal (Mean escape latency = 91.2 s on the first day and 22.55 s on the last day) and temporal (M = 92.59 s to 21.66 s) hippocampus spared groups. All lesioned groups (complete and partial)

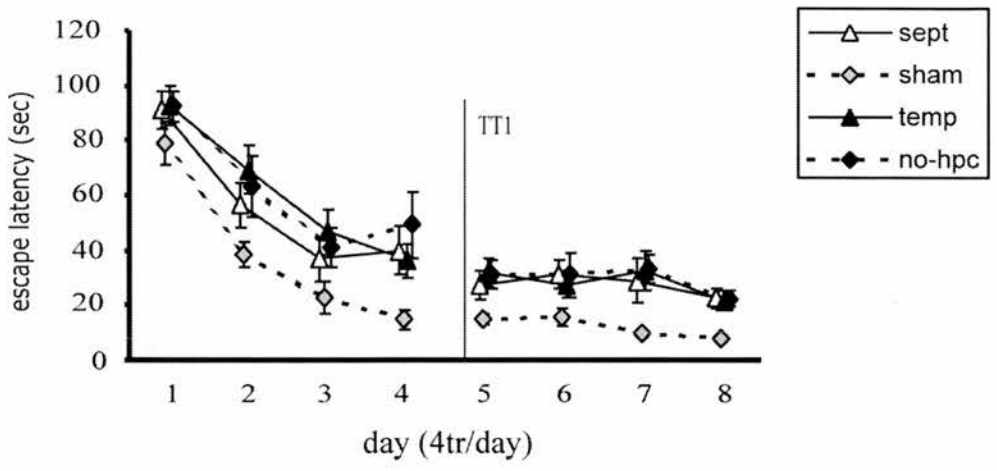

Figure 7.4: Escape latency per day. TT1: indicates when the first transfer test was given.

were significantly different from shams (M = 78.85 s to 7.83 s, $p < 0.05$). Only shams were significantly different from rats with no hippocampus spared (M = 92.35 s to 22.2 s, $p < 0.001$).

An overall analysis of the first transfer test (Fig. 7.5.a) revealed a strong effect of Quadrant ($F [2.2, 95.4] = 3.8$, $p < 0.05$; corrected for sphericity; see Methods, p.35) and no Group by Quadrant interaction ($F [6.5, 95.4] = 2963.7$, $p > 0.05$). An analysis of training quadrant only revealed a strong effect of Group ($F [3, 44] = 4.3$, $p < 0.01$). Multiple post-hoc comparisons disclosed that septal (Mean % time in training quadrant = 26.95) and temporal (M = 28.69) hippocampus spared groups were no different from each other.

An overall analysis of the final transfer test (Fig 7.5.b) revealed a strong effect of Quadrant ($F [2.3, 100.4] = 37.9$, $p < 0.001$) and a Group by Quadrant interaction ($F [6.8, 100.4] = 5.9$, $p < 0.001$). Analysis of % time in training quadrant only, revealed a strong effect of Group ($F [3, 44] = 10.8$, $p < 0.001$).

Orthogonal comparisons unveiled that rats with septal hippocampus spared (Mean % TQ = 39%) and rats with temporal hippocampus spared (M = 40.9%) did not differ from each other ($F [3, 44] < 1$) but both differed from shams (M = 55.8%; F [3, 44]

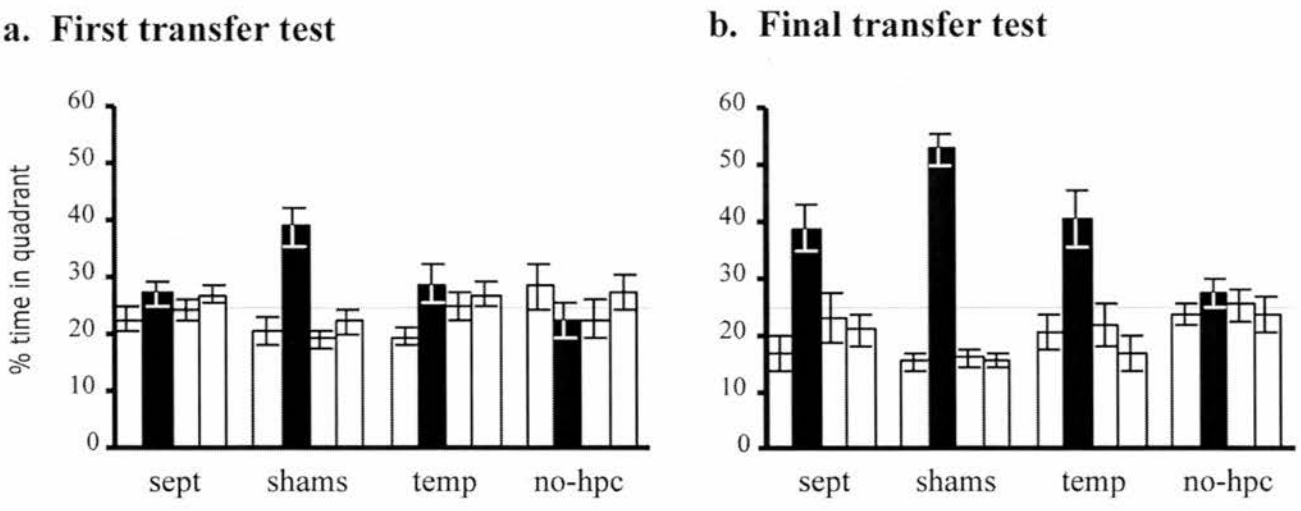

Figure 7.5: First **(a)** and final **(b)** transfer tests. Sept and temp: septal 24 to 44% and temporal 24 to 41% hippocampus spared respectively; no-hpc: no hippocampus spared. For each group: black bar represents the training quadrant, adjacent bars represent adjacent quadrants and far tight bar represents the opposite to training quadrant.

= 14.9, $p < 0.001$). However, partial lesioned rats and shams were significantly different from non-hippocampus rats ($F [3, 44]) = 14.2$, $p < 0.001$).

See Figure 7.6 for representative final transfer test swim paths.

Again no difference is found between rats with septal and temporal hippocampus spared. An analysis of other transfer test parameters was made in an attempt to disclose any measurable behavioural difference existing between these two groups.

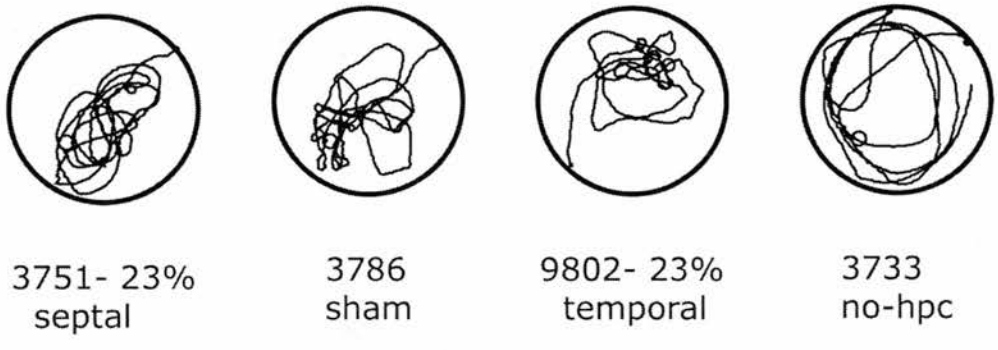

Figure 7.6: Representative swim paths taken by individual rats during the final transfer test. % is % spared tissue.

As in Chapter 5, percentage time during the first 30 seconds of the transfer test, percentage time in area, annulus crossings, first crossing and average speed were analyzed.

An overall analysis of % time in training quadrant during the first 30 seconds of the final transfer test (Fig 7.7) revealed that rats with no hippocampus are no better in the first 30 seconds than they were throughout the 60 seconds of the transfer test (M= 22.1% time in TQ), but that shams are slightly better (M= 60.8%) indicating some extinction with time. Rats with temporal hippocampus spared are identical (M= 40.56%) reflecting a fairly constant searching during the whole 60 seconds. Rats with septal hippocampus spared are surprisingly bad in the first 30 seconds of the transfer test, to the point that the bias towards the correct quadrant is very faint (M= 32.2%).

The other measurements unveiled no difference between septal and temporal hippocampus spared groups and, therefore, the data are omitted.

% time in quadrant- first 30 seconds

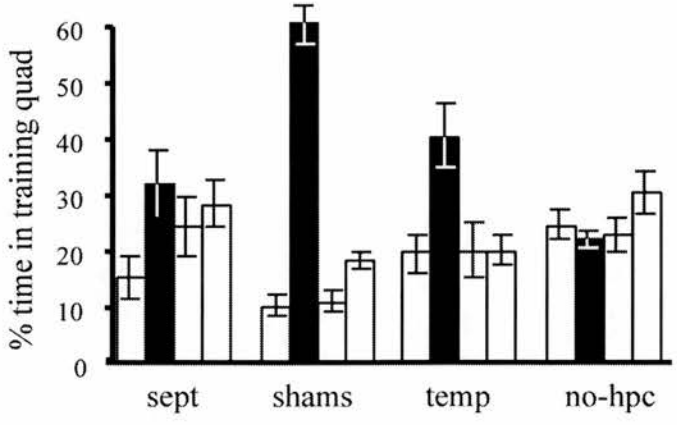

Figure 7.7: Percentage time in quadrant during the first 30 seconds of the final transfer test **(a)**. Quadrants colour and position coded as before.

7.5.3 Performance as a function of % of hippocampus spared

All the rats trained in the 1 WM Expt, including those with more than 44 % tissue spared either septally or temporally, were considered in this analysis. The following groups were obtained: rats with septal hippocampus spared (n = 12; tissue sparing ranging between 24 and 60%) and rats with temporal hippocampus spared (n = 23; sparing ranging between 24 and 80%). Figure 7.8 illustrates how performance, in terms of % time in training quadrant during the final transfer test, varies with the amount of hippocampus spared. This graph illustrates that mean performance of the septal and temporal 20-44% spared groups does not reflect the individual capacities and that, within both groups, there are animals with very high percentage times in the training quadrant.

7.5.4 Visible platform

All the rats received four trials of a visible platform task after the final transfer test. All the rats swim directly to it and reach it in less that 15 sec. This indicates that a visual impairment cannot account for deficits found in the spatial task.

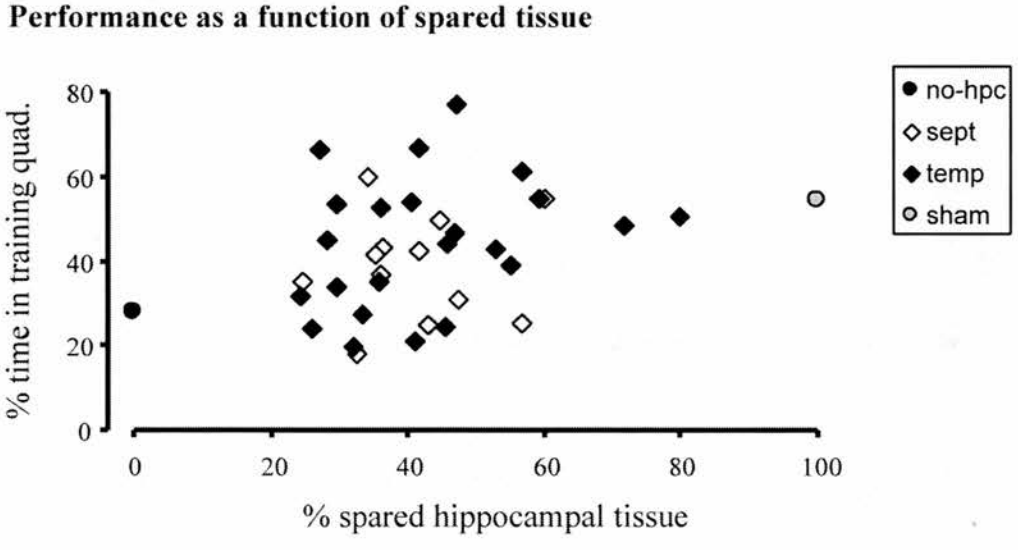

Figure 7.8: Percentage time in training quadrant during the final transfer test for individual animals as a function of amount of hippocampus spared. sept: septal hippocampus spared, temp: temporal spared, no-hpc: complete lesion. Sham and no-hpc groups are represented by the group mean.

7.6 Interim Discussion

The difference in performance of rats with temporal hippocampus spared found between the replication of Moser et al. (1995; Chapter 5) and the 2 WM Expt is clarified by the 1 WM Expt. The results obtained in the latter suggest that the reason why rats with temporal hippocampus spared displayed spatial memory in the 2 WM Expt is due to the spacing and extent of training in any watermaze, rather than the concurrent training in two different watermazes. Thus, focusing on training in one watermaze, a question this raises is why, for the temporal hippocampus spared group, one protocol (4trials/day for 8 days; total number of trials 32; one watermaze) gives rise to better transfer test performance than the other (8trials/day for 6 days; total number of trials 48; replication of Moser et al.).

7.7 Additional training of selected Groups and meta-analysis.

7.7.1 Additional training in Experiment 1

The protocols used in Chapter 5 and the 1 WM Expt above differ in two aspects: the number of days and the number of trials per day. The replication of Moser et al. (1995) was performed, in part, after the experiments presented in this chapter. For this reason it was possible to give a further 2 days of training to some of the sham lesioned rats (2 out of 19) and some of the temporal hippocampus spared rats (5 out of 10) included in Chapter 5. The protocol was matched to that used in the previous days and, therefore, consisted of 8 trials per day in 2 sessions of 4 trials starting immediately after the 'final' transfer test done on day 7. A further transfer test was then given one day after this extra training (day 9). The prior performance of these 7 animals was representative of their group. Figure 7.9 shows that the 5 rats with temporal hippocampus spared acquired a bias for the correct quadrant ($51 \pm 9.7\%$; $t = 2.673$ in comparison with chance level, $p = 0.05$). Shams reached a training quadrant score of 79.4%.

Day 9 transfer test, added to replication of Moser et al. (1995)

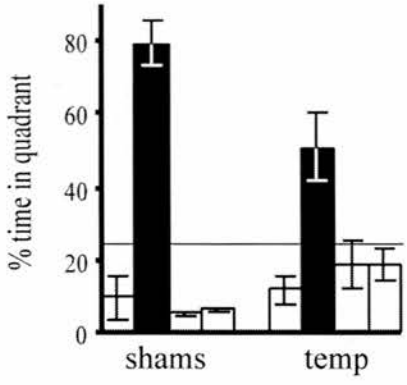

Figure 7.9: Performance during additional transfer test (day 9) given to rats trained originally with an identical protocol to that of Moser et al. (1995) and presented in Chapter 5. Temp: temporal 20 to 41 % hippocampus spared. Quadrants colour and position coded as before.

7.7.2 Meta-analysis

The results of the three experiments and additional training included in Chapter 5 and in this chapter, although apparently inconsistent with each other, suggest after further analysis that the different outcomes are a result of the training protocols used and may not reflect a real disagreement.

The results of all the three experiments were plotted together (Figure 7.10) as % time in training quadrant during the transfer tests (averaged across the three experiments for each group) against either number of trials of training, or number of days. Detailed statistical analysis of within-subject trends cannot be done as the numbers of animals per data point vary through the resulting graphs. The amount of hippocampus spared in each of the partial lesioned groups is equivalent across experiments. Rats with no-hippocampus spared were at chance in all experiments and so not considered in this analysis, while the sham lesioned rats are plotted as 'inserts' in the figure.

When plotted as a function of the number of trials of training, the performance of rats with septal hippocampus spared show a monotonic function whereas those with temporal hippocampus spared do not (Fig 7.10 left). However, when the data are re-

grouped and plotted as a function of number of days of training, the rats with temporal hippocampus spared show a monotonic function (Fig 7.10 right; in reading Figure 7.10, it is important to recognise that this is a 'meta-analysis' and that the downward portions of either set of data do not reflect worsening performance with overtraining). Also, it is apparent that rats with temporal hippocampus spared are not above chance before day 8, independently of the training protocol, and that septal and temporal spared rats reach the same level of performance on this day. The plot of performance by trials suggests that equivalent levels of performance would have been reached after 64 trials had septal spared rats been given this additional training.

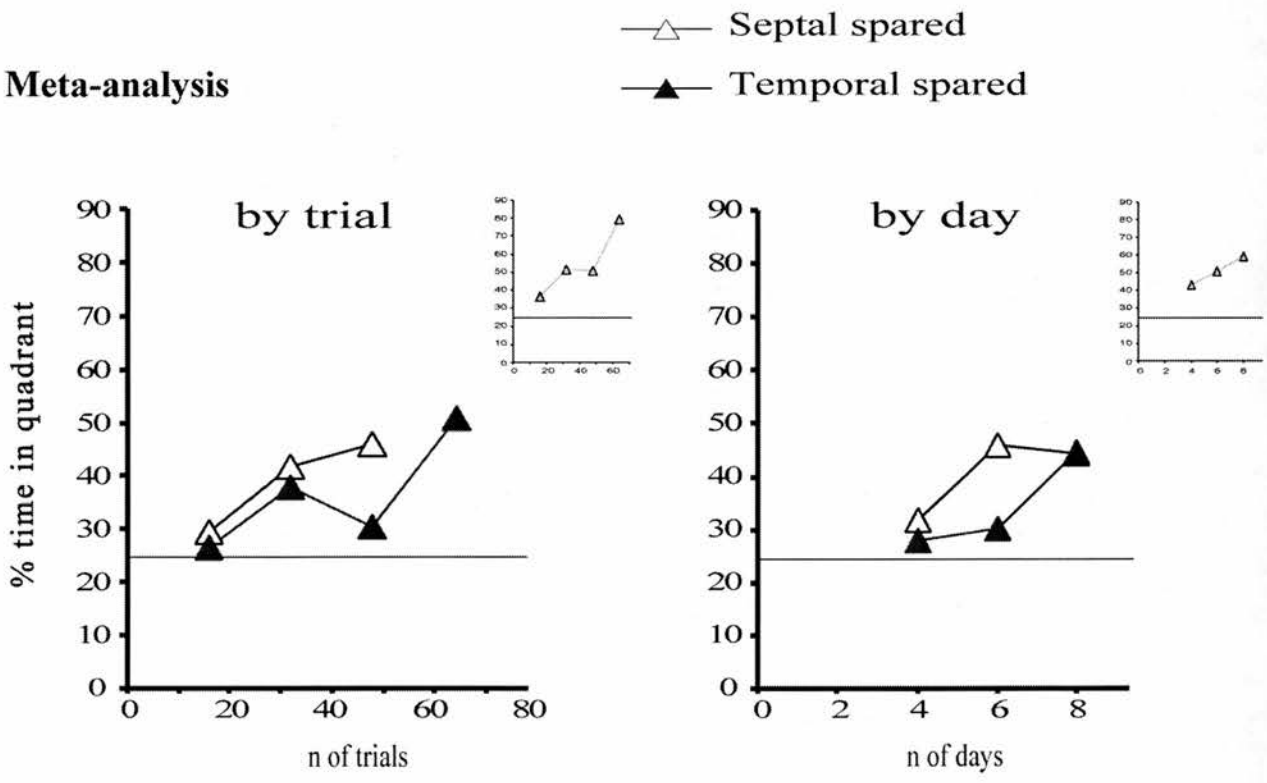

Figure 7.10: Percentage time in training quadrant during transfer test averaged across groups as a function of number of trials or number of days given immediately prior to a given transfer test.

7.8 Discussion

The key finding of this series of experiments is that rats with temporal 20-44% hippocampus can learn an allocentric spatial reference memory task with certain training protocols. The experiments presented in this chapter and Chapter 5 establish that Moser et al's (1993, 1995) finding of poor learning in such animals can be replicated, but the implication that has been widely drawn from their results to the effect that septal and temporal hippocampus may differ in function could be an overinterpretation. Changing the training protocol to one that increased the number of days of training, rather than the number of trials, favoured lesioned animals with temporal hippocampus spared.

These findings will be discussed in relation to (1) the performance of animals with temporal hippocampus spared; (2) the existence of subtle rather than major differences in the contribution of septal and temporal hippocampus to spatial learning; (3) the two hypotheses on which these experiments were based; (4) implications for other theories of hippocampal function.

7.8.1 Temporal hippocampus involvement in spatial memory.

The first point to clarify about the successful learning of the one and the two watermaze tasks by rats with only the temporal 20 to 40% of hippocampus spared is that it is not merely a consequence of 'overtraining'. First, rats with no hippocampus spared are at chance in all transfer tests indicating that above chance performance is hippocampus-dependent. Second, the performance of rats with only temporal hippocampus spared was better in the one watermaze experiment (with only 32 training trials) than in the replication of Moser et al. (1995; with 48 trials). Third, if the above chance performance of rats with temporal hippocampus spared was due to 'overtraining', one might expect rats with septal hippocampus spared to be even better after such training if a septo-temporal gradient of spatial learning capacity existed. Contrary to this, in the one watermaze experiment, the level of performance of both septal and temporal hippocampus spared rats was identical after 8 days and did not differ throughout training.

Interventions in the temporal hippocampus have been associated with spatial deficits before. These data are discussed with the unitary hypothesis further down.

The use of two concurrent watermazes reveals some interesting facts. Interference between the two contexts, the need to process more information and to select the correct one in each situation and the necessity for a bigger storage capacity are some of the reasons why learning two concurrent watermazes could be expected to be more demanding than learning just one. However, acquisition seems to be facilitated by two concurrent watermaze training in all the groups, except that with no hippocampus spared. In the sham group, the mean escape latency for the first 4 trials (average of first session across watermazes) is 59.7 seconds. This is lower than the mean escape latency for the first 4 trials in one pool (one watermaze experiment), which is 78.8 seconds. However, comparison of transfer test performances on day 9 between the two and the one watermaze experiments reveals that rats trained in just one watermaze perform better (% time in training quadrant = 55.7 %) than rats trained in two concurrent watermazes (47.3 %).

During acquisition, rats with partial lesions display a similar pattern to that of shams. In the 2 WM Expt their latencies go from 73.5 s on the first day to 11.7 s on the last day for rats with temporal hippocampus spared, and from 75.2 s to 12.9 s for rats with septal hippocampus spared. Latencies in the 1 WM Expt are comparatively longer going from 92.59 s to 21.6 s and from 91.2 s to 22.5 s for the respective groups. However, during the final transfer test performance is similar across experiments.

The initial facilitation resulting from the concurrent training is likely to depend on the learning of generic task-dependent skills (learning to climb onto the platform or to swim away from the walls), that can be applied to either pool, rather than on the learning of the spatial layout of the environment and its significance.

The facilitatory effect on latency during acquisition of the concurrent training is not observed in rats with complete hippocampal lesions, which are similar across experiments, and slower than the other groups in the 2 WM Expt. This does not necessarily mean that learning the procedural aspects of the task is hippocampal

dependent. Facilitation of procedural aspects is likely to be more efficient when combined with a capacity for spatial learning.

Another issue that becomes apparent is that learning two concurrent watermazes does not require more volume of hippocampus than the minimal amount known to be sufficient to learn one watermaze. This raises a very interesting point when it is considered that, in Chapter 6, it became obvious that learning to flexibly use the spatial information in a given environment (delayed match to place task) did require more tissue than the minimum necessary to learn one fixed relevant position (reference memory task) in the same environment. The comparison between the present findings and those of Chapter 6 further supports the idea that the hippocampus does not merely store a map of an environment but also the behavioural information that goes with it. Thus, encoding two spatial environments does not require more hippocampus than encoding just one, probably, because the behavioural information is identical for both, i.e. a given location is relevant and provides reward. This is consistent with findings that hippocampal cells encode for behavioural, as well as spatial, information (Wood et al., 1999; Hampson et al., 1999a).

Analysis of performance in the 1 WM Expt as a function of % tissue spared septally or temporally reveals that, at least in the temporal spared group, the more tissue spared the better the mean performance. However, it also reveals that the lower mean percentage time in training quadrant in the group of rats with little hippocampus spared is a result of the variability in that group rather than of a general decrease in persistence in the training quadrant. Some of the subjects in the group with smaller amounts of hippocampus spared performed as well as shams.

7.8.2 Differences between septal and temporal hippocampus spared rats in a spatial task.

An additional finding was that the level of performance reached by rats with septal hippocampus spared seems to be more responsive to the total number of trials of training, whereas that of rats with temporal hippocampus spared is more sensitive to number of days of training (Fig 7.10). It seems that a protocol with a high number of

trials per day is more favourable for rats with septal hippocampus spared and less so for rats with temporal hippocampus spared. If this pattern can be extrapolated beyond the particular protocols used here, it might be that a protocol of as few as 1 trial per day would favour rats with temporal hippocampus spared. In this circumstance, performance by such a group might reach above chance earlier than in a group with septal hippocampus spared.

But the main question remains, why might having more days of training be more favourable for rats with only temporal hippocampus spared than having more training sessions spaced within the day? Where does the major determinant of learning rate reside? To reach above chance performance rats with temporal hippocampus spared require a smaller number of trials when the training sessions (4 consecutive trials) are spaced by 24 hours (one watermaze experiment) than when they are given twice a day (replication of Moser et al., 1995). This suggests that in this group, the length of the consolidation period (considered here as the period between two training sessions) is relevant for the final level of performance.

This difference in spatial learning rate between rats with septal and temporal hippocampus spared and the possibility that it resides in different speeds of memory consolidation is discussed below in terms of differences in the intrinsic and extrinsic circuits along the septotemporal axis and also with respect to the possibility that they are the result of a lesion artefact.

Rats with temporal hippocampus spared differ from rats with septal hippocampus spared in aspects of their intrinsic circuit (Fig. 7.11.a). Although the intrinsic architecture of the hippocampus is homogeneous along the longitudinal axis, certain quantitative aspects of the circuit vary along this axis. One example is the ratio between number of DG granular and CA3 pyramidal cells, as a result of which, the mossy fibre projection is convergent in the septal hippocampus but divergent in the temporal hippocampus (Amaral and Witter, 1995). Another example is the evidence presented by Li et al. (1994) that all the CA3 cells in the temporal hippocampus have a backward projection to DG, but only a few in the septal hippocampus do. Also, in the dentate gyrus, the ratio between number of granular cells and basket cells varies

along the septotemporal axis (Seress and Pokorny, 1981) which has been suggested to result in more inhibition in the temporal pole. It is not known how these differences affect memory processes, but it is not unreasonable to suggest they could. Specifically, they could affect the memory consolidation process.

Touching on a different aspect of the intrinsic circuit, neuromodulatory transmitters are found in higher concentration in the temporal hippocampus. This is true of dopaminergic projections (Verney et al., 1985; Febvret et al., 1991), enkephalin (Gall et al., 1981), substance P, oxytocin (van Leeuwen et al. 1985) and vasopressin (Caffé et al., 1987). It is possible that these molecules have an effect in consolidation processes and therefore affect consolidation in the temporal and the septal hippocampus differently.

Although differences in the processing circuit are likely to result in different learning rates, the septal and the temporal hippocampus also differ in the layout of their extrinsic circuit (Fig. 7.11.b) and it is reasonable to expect these differences to contribute to the unequal learning rate.

The septal hippocampus is the main recipient of projections reaching the hippocampus from areas of the entorhinal cortex (EC) that are innervated by pathways from neocortical sensory areas (Burwell et al., 1995; Burwell and Amaral, 1998a and b; Dolorfo and Amaral, 1998a and b). The temporal hippocampus, on the other hand, is reciprocally connected with the amygdala and projects to the hypothalamus (Jay et al.,1989; van Groen and Wyss, 1990; Köhler et al. 1985). The reference memory task is generally understood to depend on the learning of sensory information and its significance. Also, the watermaze rooms are carefully laid out to be heterogeneous in terms of sensory cues (so that navigation is possible) but non-stimulating in aspects that could be considered to be of emotional importance such as lighting, temperature, loud sounds and position of the platform with respect to the home cage.

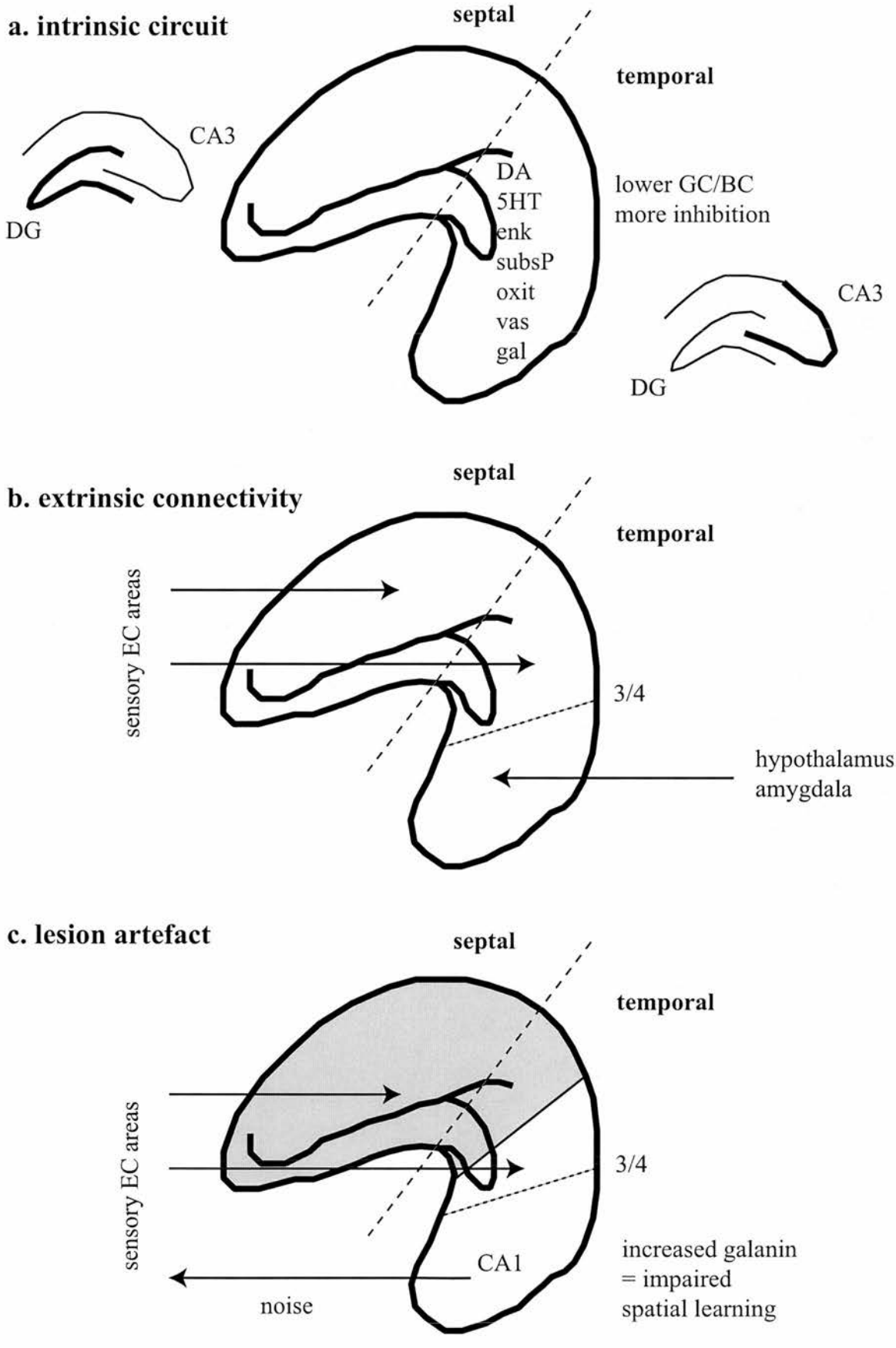

Figure 7.11: Schematic drawing of differences between the septal and the temporal poles of the hippocampus in the intrinsic circuit (a), the extrinsic circuit (b) or in the reaction to the lesion (c), that might be the reason for the subtle behavioural differences observed between the behaviour of rats with septal or temporal hippocampus spared. See text.

For this reason it might seem surprising that rats that are receiving little input from sensory driven structures (temporal spared group) are capable of learning this task. In this group, the amygdalar input is intact, but how can this input, generally understood as modulatory, help spatial learning? Examples of modulation of hippocampus function by the amygdala have been widely reported. For example, although lesions to the amygdala do not generate impairments in the watermaze (Sutherland and McDonald, 1990), this structure modulates acquisition of this task by the hippocampus (Packard et al., 1994; Packard and Teather, 1998). Hippocampal LTP is also modulated by the basolateral and basomedial amygdala (Ikegaya et al., 1996 and 1997). However, the amygdala not only modulates hippocampal function, it is also crucially involved in different memory processes (Pitkänen et al., 1997; Killcross et al., 1997; Maren, 1999) such as detection of stimuli of emotional relevance and development of appropriate responses (Rogan and LeDoux, 1996) or establishment of stimuli reward associations (Ehlers et al., 1998). In fact a double dissociation of hippocampal and amygdalar function in memory is reported by Bechara et al. (1995) in humans. Additionally, the amygdala is reciprocally connected with the perirhinal and parahippocampal cortices in the monkey (Stefanaci et al., 1996) from which it receives sensory information. These data suggest that the reciprocal connection between the amygdala and the temporal hippocampus are not just modulatory and that they could play a role in the capacity of temporal spared rats to develop appropriate spatial responses in relation to a reward (platform) in the absence of the septal hippocampus.

However, a more important role might be played by the projection from neocortical sensory areas to entorhinal DG-projecting bands terminating in the temporal hippocampus. For example, olfactory cortices project to the whole extent of the entorhinal cortex (EC), with the exception of the caudal MEA (which projects to septal DG). Thus, the olfactory input to the temporal hippocampus conveyed via the EC is intact. The olfactory system is well developed in the rat. However, little is known about the use of olfactory cues in the watermaze.

The temporal hippocampus is also differentially connected to the prefrontal cortex, a structure known to be involved in response planning but also in episodic memory. It

is possible that navigation is facilitated through the influence of the hippocampus over the prefrontal cortex.

Evidence has been presented suggesting that partial lesions to the hippocampus respect the general aspect of the circuit in the spared tissue and the extrinsic inputs to this area (Chapter 3). Also, Moser et al. (1995) presented evidence that a partial lesion of the hippocampus did not affect acetylcholinesterase staining or different electrophysiological measures in the spared part of the structure. However, it is possible that an ibotenic acid lesion affects the two partially lesioned groups differently for reasons other than functional and that compensatory mechanisms take longer to have an effect when the temporal hippocampus is spared. Rats with temporal hippocampus spared are at chance up to the 6th day of training. However, after 8 days the level of performance reached is correlated with the number of trials given per day (replication of Moser et al.: 8 trials per day, 51% in training quadrant; two concurrent watermazes: 4 trials per day in each watermaze, 41%; one watermaze: 4 trials per day, 38%). This pattern suggests that there is some kind of threshold, met on day 6, after which these rats respond to both trials and days and, thus, support the idea that this group of rats is compensating for the effects of the lesion and manages to do so after 6 days of training. Possible explanations are illustrated in Figure 7.11.c and explained below.

Galanin is a neuropeptide for which there are 4.8 times more receptors in the temporal than in the septal half of the hippocampus (Valkna et al., 1995). Accordingly, its effects are more patent in the temporal hippocampus (Robinson et al., 1996; Consolo et al., 1998; Ogren et al., 1998). Behaviourally, galanin has been associated with reduction of performance in different working and spatial memory tasks (McDonald et al. 1998). In fact, galanin infused into the ventral hippocampus impairs spatial learning while infused into the dorsal results in amelioration (Ogren et al., 1998). Entorhinal cortex lesions were found to induce an increase in galanin in the dentate gyrus (Harrison and Henderson, 1999), a result that raises the concern that galanin release might be increased in the spared area of tissue after a partial lesion to the hippocampus. According to the data above, this increase would affect mainly the temporal hippocampus where it would contribute to deficits in the initial

stages of learning. This hypothesis could be tested by injecting a galanin antagonist into the ventral hippocampus after lesioning the septal part of the structure.

Another possible lesion artefact could result from the fact that the temporal and proximal CA1 region projects to areas of EC which in turn project to the septal DG (Amaral and Witter, 1995). As this is lesioned in temporal spared rats it could be argued that the dead-end temporoproximal CA1 to EC projection generates noise. It is possible then that during initial training the circuit is learning to compensate for this imbalance.

Several possible explanations as to why rats with temporal hippocampus spared are impaired during the initial stages of learning have been given. Although one could see the utility of having two different consolidation speeds within the hippocampus (the efficiency of such system has been highlighted before by Murre (1996) in relation to hippocampus and neocortex) it is difficult to relate this fact with what is known about the extrinsic and intrinsic circuits of the hippocampus. Although septotemporal differences in quantitative aspects of the intrinsic circuit are likely to result in differences in a consolidation process, the segregation of extrinsic connections along the septotemporal axis must be taken into account as well. The most likely explanation to the subtle differences in learning rate observed between rats with septal and temporal hippocampus spared probably resides in a combination of the different aspects highlighted in Fig 7.11.

Richmond et al. (1999) also found that rats with temporal hippocampus spared were able to learn a reference memory task in the watermaze.

Surprisingly their protocol consisted of 6 days of training at a rate of 4 trials per day. According to what has just been discussed, this protocol should have resulted in chance performance by this group because, although it involves four trials per day, it is only given for 6 days. I did not perform a transfer test after 6 days of training in the 1 WM Expt (also 4 trials per day), but performance after 8 days of training was 41 % time in training quadrant. In Richmond et al. (1999) study performance reached by the temporal spared group after 6 days of training is at around 45 % time in training quadrant. However, in this study all the rats had been previously exposed to

a contextual fear conditioning task over 4 days. It is possible that this previous experience of a task that was, additionally, found to be more sensitive to temporal hippocampal lesions, helped rats with this part of the hippocampus spared in the consequent watermaze learning. If this were true, it would support the idea that activation of the temporal hippocampus in partial lesioned rats facilitates learning rate even when the activation is the result of previous exposure to an environment and experimental requirements different from the ones consequently tested. This notion is, nonetheless, not supported by a parallel study by Bannerman et al. (1999) in which rats with temporal hippocampus spared are impaired in a reference memory task in the watermaze trained with an identical protocol of 4 trials per day for 6 days. These rats had also been trained in an elevated T-maze and a DRL task and observed in a photocell chamber previous to the watermaze training. It is only speculation to suggest that the reason why, in this case, previous exposure to other tasks did not result in better performance by this group could be related to the fact that none of these tasks differentially required the temporal hippocampus.

Returning to this chapter's results, despite the difference in learning rate found between rats with septal and temporal hippocampus spared, no difference was found in other behavioural measures taken during transfer test performance, such as swimming speed, first crossing and annulus crossing. Specifically, swimming speed was equivalent in both groups in both 2 WM Expt and the 1 WM Expt. Furthermore, no difference in swimming speed was found in Chapter 5, even though in the replication of Moser et al. (1995) performance in terms of % time in training quadrant was impaired in the temporal hippocampus spared rats. This result goes directly against the findings of Bannerman et al. (1999) that lesions to the temporal, but not the septal, hippocampus result in increased locomotor activity measured as swimming speed during a transfer test in the watermaze. A result that led the authors to conclude that a double dissociation had been found by which septal hippocampal lesions impaired spatial memory and temporal lesions impaired locomotor control. Regardless of whether swimming speed can be considered a hippocampus-dependent role, the results presented in this thesis, so far, do not support this idea.

7.8.3 The two hypothesis

Two possible hypothesis were put forward in the introduction to this chapter.

The first of them, was the functional differentiation hypothesis proposed by Moser and Moser (1998b) and evidence for which was presented in Chapter 5. Here this hypothesis is discussed more thoroughly and evidence against it is included.

The second hypothesis introduced interprets the differences along the septotemporal axis as part of a single hippocampal process. It is also discussed further below.

7.8.3.1 The functional differentiation hypothesis

There are, undoubtedly, many advantages in a functional differentiation along the longitudinal axis. Present, and often controversial, theories of hippocampal function would result in complementary, and no longer controversial, theories if their anatomical ground was found to be secluded to different areas of the hippocampus.

A thorough review on the evidence that supports the functional differentiation hypothesis was presented in Chapter 5 and will, therefore, be omitted here.

The findings presented in this chapter that both septal and temporal parts of the hippocampus can sustain spatial learning do not, however, support this hypothesis. Additionally, the direct implication of the hypothesis, that it should be possible to find a double dissociation (i.e. a temporal, but not septal, hippocampus dependent non-spatial task) lacks any experimental evidence so far. Studies that have followed this line of thought fail to find a non-spatial temporal hippocampus dependent task. Context fear conditioning has been chosen because of the differential connection between the temporal hippocampus and the amygdala and the known involvement of the latter in this task. Richmond et al. (1999) found that some measures of a context fear conditioning task were differentially affected by lesions to the ventral hippocampus. Although these data would, in principle support the functional differentiation hypothesis, within subject comparisons revealed that dorsal hippocampal lesioned rats were unimpaired in a six day watermaze reference

memory task. Thus, no within subject double dissociation was found. Other studies (Ferbinteanu and MacDonald, Soc. Neurosci. Abstr. 649.19, 1999 and Tuvnes et al., Soc. Neurosci. Abstr. 649.3, 1999) also fail to find a difference between dorsal and ventral lesions to the hippocampus in a context fear conditioning task. Although it is possible that choosing between environments is an inherently spatial manoeuvre, freezing, which is known to be modulated by the amygdala, should be differentially affected. However, septal hippocampus lesions have been found to affect context fear conditioning in other laboratories (Phillips and LeDoux, 1994; Maren and Fanselow, 1997), although this effect can be seen only after 21 days, but not 7, in circumstances in which the rat had been exposed to the context previous to the shock episode (Winocur, 1985). The conditions in which contextual fear conditioning becomes hippocampal dependent and whether context learning could be interpreted as an inherently spatial process is another matter that will not be discussed here.

In another study, an internal cue-shock task (non-spatial) was chosen as the possible ventral dependent task (Hock and Bunsey, 1998). Here rats had to learn whether particular internal states (degree of hunger) predicted a shock or not. Detection of such type of internal states have been suggested to depend on the hippocampus. For example, H.M.'s capacity to detect whether he is hungry or not is impaired (Hebben et al., 1985). Also, Davidson and Jarrard (1993) determined that, although normal rats were capable of learning relationships between internal cues and particular outcomes (Davidson et al., 1992) even when the latter did not change the internal state (for example, they did not satiate the hunger), lesions to the hippocampus impaired this ability. As the temporal hippocampus is differentially connected to the hypothalamus, parts of which are involved in homeostasis and are, therefore, essential for detecting internal states, it was believed that the fore mentioned task would differentially require this part of the hippocampus. However, no difference was found between rats with septal or temporal hippocampus spared (Hock and Bunsey, 1998). Similarly, Hock et al. (Soc. Neurosci. Abstr. 40.3, 1999) reported no difference between septal and temporal hippocampus spared rats in associating a particular odour with a hunger estate.

No double dissociation was obtained when performance of partial lesioned rats were measured in a DRL task, a non-spatial task that requires detection of time, and compared with that of rats trained in a spatial task, a watermaze and a T-maze task (Bannerman et al., 1999). The DRL task was equally affected by rats with septal or temporal hippocampal lesions. This is an interesting result when considered together with previous studies (Ellen et al., 1964; Haddad and Rabe, 1967 and Grant and Jarrard, 1968) suggesting that ventral but not dorsal hippocampal lesions affected DRL performance. As the lesions in Bannerman et al. (1999) are of extremely good quality, the findings of the previous studies might be the result of uneven lesions with extra-hippocampal damage, an issue that was already discussed in Chapter 4 in relation to the controversial findings obtained during the 60s and 70s.

Lorenzini et al. (1996, 1997) found that both TTX inactivation of the dorsal or the ventral hippocampus impaired passive avoidance response. However, injections at different times after acquisition affected the dorsal or the ventral hippocampus differently such that inactivation of the ventral hippocampus was not effective when 1.5 hours had elapsed. It is claimed that the ventral hippocampus has a shorter period of consolidation. If the experiment could be compared considering the technical differences, this result would not conform with the finding that a longer interval between watermaze sessions is more favourable to rats with temporal hippocampus spared.

A direct prediction for a double dissociation is found in the anatomical study by Risold and Swanson (1996), who affirm that septal levels of the rodent hippocampus influence female sexual behaviour while temporal levels influence male sexual behaviour. However, no behavioural studies have been done, to my knowledge, to test this anatomical finding.

Additionally, there is no evidence that spatial and non-spatial functions are incompatible. In fact the septal part of the hippocampus has been associated with non-spatial forms of memory before. Social interaction is very sensitive to anxiety and, thus it decreases or increases as the circumstances are changed from anxiogenic to anxiolytic, respectively. An anxious response to an unfamiliar environment could

be considered to be an emotional driven behaviour and, therefore, to be dependent on the temporal hippocampus. However, nicotine receptors in the dorsal hippocampus were found to modulate this response (File et al., 1998). Also, social transmission of food preference, which because of the social and ingestive components would constitute a good candidate for a temporal hippocampus dependent task, is found to be affected by lesions to the dorsal hippocampus (Roberts and Shapiro, Soc. Neurosci. Abstr. 253.2, 1999). Cells in the dorsal hippocampus were also found to respond to non-spatial aspects of a DMS task (Hampson et al., 1999a). Finally, the posterior (or septal in rats) hippocampus of human subjects was differentially active during a task that required verbal episodic memory (Fernandez et al., 1998). It is true, however, that verbal memory in humans is considered by O'Keefe and Nadel (1978) and, later, by O'Keefe (1999), as an extension of the spatial capacity of the hippocampus.

Thus, although lesions to the septal part of the hippocampus consistently impair spatial memory, the septal hippocampus is also found to modulate and respond to non-spatial memory components.

From the anatomical point of view, Moser and Moser (1998b) have put a lot of emphasis in the fact that the projection from the perirhinal and postrhinal cortices to the hippocampus (via EC) terminates mainly in the septal hippocampus and have considered this a strong anatomical support of their functional differentiation hypothesis. However, not only does this projection also terminate in the temporal hippocampus (Burwell, 2000), but axon-sparing lesions to the perirhinal and postrhinal cortices do not impair performance in a watermaze task (Bussey et al., 1999) or in other spatial tasks (Bussey et al., 2000). This lack of involvement of the perirhinal cortex in spatial memory is also suggested by c-fos activation studies (Wan et al., 1999). All together this evidence suggests that peri and postrhinal inputs are not crucial for spatial memory.

There is evidence that the thalamus and mammillary body, on the other hand, do play an important role in spatial memory (Neave et al., 1997; Warburton and Aggleton, 1999; Mumby et al., 1999b). As discussed in Chapter 4, the projections from the

nucleus reuniens to the hippocampus reach both the septal and temporal levels and the efferents from hippocampus into the anterior thalamus and mammillary bodies originate mainly in the temporal subiculum. As a whole these data do not support the functional differentiation hypothesis.

7.8.3.2 The unitary hypothesis

The results obtained in the two experiments presented in this chapter, that both ends of the hippocampus can support spatial memory, support the hypothesis that this structure is involved in one memory process. It was expected that a more demanding task (acquisition of two concurrent watermazes) would engage the temporal hippocampus such that both septal and temporal parts of the structure would be required to learn the task. Yet it was found that either of them was sufficient not only in this task, but also when learning one watermaze at a lower trial/day ratio.

According to this hypothesis the whole of the hippocampus is responsible for one type of memory process.

Behavioural evidence supporting the unitary hypothesis resides in the fact that interventions in the ventral hippocampus have been associated with deficits in spatial learning elsewhere. Infusion of galanin into the ventral hippocampus of rats 20 minutes before training impairs acquisition of a spatial task (Ogren et al., 1996; Schött et al., 1998). Acquisition of a delayed and a non-delayed spatial cued radial-arm task is impaired by temporary inactivation of the ventral CA1/subiculum (Floresco et al., 1997). Infusion of nicotine in the ventral hippocampus facilitates performance in a working memory task in a radial-arm maze even though lesions to this part of the structure have no effect (Levin et al., 1999). Also, Vann et al. (2000) find c-fos activation in the temporal, as well as the septal, hippocampus of rats trained in a spatial radial arm maze. Only when the rats are required to perform the task in a new environment is the dorsal hippocampus differentially activated.

Evidence suggesting that the temporal hippocampus is capable of supporting spatial memory in the absence of the septal hippocampus is also found. Complete, but not dorsal, hippocampal lesions are found to impair working memory for allocentric

spatial distances (Long and Kesner, 1998) suggesting that the ventral hippocampus is capable of supporting memory for spatial information. Similarly, rats with only the ventral hippocampus spared were capable of developing a bias for the training quadrant in a reference memory task in the watermaze (Richmond et al., 1999; and results presented in this chapter).

Also, Jung et al. (1994) and Poucet et al. (1994) both found place cells in the temporal hippocampus. These two studies differ in that the former finds that the size of the place fields of these cells is generally larger than that of cells in the septal hippocampus, while the latter finds no difference in place field sizes. Colombo et al. (1998) also found cells with spatial responses in the anterior hippocampus of monkeys performing a delayed matching to place task. However, the number of cells with this type of response was smaller than that found in the posterior (septal in rats) hippocampus.

In my view, the intrinsic circuit is more determinant of the processing capacity of a structure than the extrinsic circuit. While the former defines the type of algorithms that are possible, the latter determines the particular information that is processed by that algorithm. The basic architecture of the intrinsic circuit is homogeneous along the hippocampal septotemporal axis. This constitutes anatomical evidence for the unitary hypothesis. Moreover, both CA3 associational projections and Schaffer collaterals extent along the longitudinal axis and, consequently, spread information outside the lamella and across septotemporal borders. In this situation, the segregation of extrinsic inputs along the septotemporal axis is lost as information moves along the transverse axis.

This kind of circuit has the potential for comparison between segregated inputs that can then be processed together. This capacity might be key in the functional capacity of the hippocampus. Episodic memory (Tulving, 1993; Morris and Frey, 1997; Vargha-Kahdem et al., 1997) is one example in which the need for a system that can integrate different types of information becomes apparent. Episodic memories are made of unique combinations of stimuli of very different nature. These stimuli can be spatial as well as non-spatial and often have a very strong emotional component.

Because the different elements of an episodic memory have different peripheral origins (sensory versus internal state for example) it is not surprising that they enter the hippocampus through different fibre systems and at different septotemporal levels. It is in these circumstances that the utility of an intrinsic associational system, crossing over the septotemporal boundaries, is highlighted, as the memory only acquires its full significance when the different components are considered together. Thus, by testing septal or temporal hippocampal lesioned animals in tasks that rely mainly on one memory component one might find support for the functional differentiation hypothesis but one might also be discarding an essential asset of the hippocampus, i.e. its capacity to integrate information from different origins.

For this reason, although there are, undoubtedly, benefits in finding a double dissociation between the septal and the temporal hippocampus, my concern is that focusing independently on each pole of the hippocampus would deviate the attention from an important issue, which is the capacity of the hippocampus to integrate different kinds of stimuli according to their spatial and temporal relationships.

Segregation of inputs and/or outputs along the septotemporal axis is a fact that needs more careful consideration. For example, connections between the temporal hippocampus and the hypothalamus have been used to support the fact that the temporal hippocampus might be involved in non-spatial types of memory. The hypothalamus is involved in homeostasis in general and, as such, has been associated with social, sexual, ingestive and defensive behaviours. However, it is difficult to think of a better reason (if evolution could reason) to develop a system capable of encoding spatial information than to give a spatial frame to those types of basic behaviours. For example, the hypothalamus is associated with urinating responses or suppression of them. It is well known that mammals mark their territories with urine and faeces and, thus, for an animal to be able to control those responses in association with the space is essential. Similarly, ingestion is very closely associated with space as certain foods can only be found in certain places. Evidence for relationships between spatial memory and hypothalamic function exists. Activation of the hypothalamus-pituitary-adrenocortical-axis, for example, modulates the differential effects of chronic stress over hippocampus-dependent spatial memory

(Ohl and Fuchs, 1999). The hypothalamus is known to modulate food intake (Hagan et al., 1998), which in turn facilitates performance in the watermaze (Oomura et al., 1993).

The temporal hippocampus is also differentially connected with the amygdala. The same line of thought is applied here, when the importance of remembering a place associated with fear is highlighted. Thus, sensory information is what determines the space and is, undoubtedly, essential, but the encoding of spatial information can also be influenced by emotional information and, conversely, is likely to influence emotional and hypothalamic dependent behaviours.

Moreover, the septal hippocampus also known to influence hypothalamic function, through inputs to the hypothalamus via the lateral septum (Risold and Swanson, 1996, 1997). Similarly the amygdala is known to modulate the effect of drugs injected into the dorsal hippocampus (Roozendaal and McGaugh, 1997; Lorenzini et al., 1996).

The latter opens an interesting point. While the amygdala does not project to the septal hippocampus it is still capable of modulating the effects of drugs injected into this part of the structure. It is possible that temporal levels of the hippocampus act as intermediaries in this effect. Levin et al. (1999) found that, although lesions to the ventral hippocampus did not impair working memory in a radial maze, injection of nicotine into this part of the structure facilitated performance in this task. Both these results suggest that intrinsic connections along the septotemporal pole influence the outcome of local processing. This brings me to the next point. The evidence described so far suggests the worrying possibility that partial lesions to the structure might not be the optimal way of approaching the subject of differences along the septotemporal axis. Partial lesions have the advantage that they disrupt the normal internal flow of information within the hippocampus, restricting it to a lamellar sense. This permits the study of the capacity of one part of the structure without the influence of the other. However, they could also lead to misinterpretations, as the septal and the temporal poles of the hippocampus do not naturally act independently. Evidence for this can be found, for example, in the finding by Chiba et al. (Soc.

Neurosci. Abstr. 1992) that, although either septal or temporal hippocampal lesions spare performance in the long spatial intervals of a serial position task, complete lesions (Chiba et al., 1994) abolish memory for these long intervals.

Key questions in relation to this hypothesis remain to be answered such as how do the different segregated inputs and outputs contribute to a single function and how are the different types of information integrated.

7.8.4 Relevance to other theories of hippocampal function

My results suggest that although the whole hippocampus is involved in spatial learning the septal and the temporal poles do so in different ways which are dependent on the training protocol. In all three experiments the rats have to face a similar problem for which they are given similar information. Spatial learning is therefore not only dependent on the task itself but on how the task is learnt. Although of a different nature this can be compared with what happens in other types of tasks such as CS-US associations in which the presence or absence of contextual information or the specific temporal pattern of the association can make the task dependent or independent of the hippocampus (Phillips and LeDoux, 1994; Good et al., 1998).

Second, it highlights the fact that with very little sensory (olfactory, visual, auditory and polimodal) information, as implied by the anatomical data described above, rats with temporal hippocampus spared can learn a spatial memory task. Terminals specific to this part of the hippocampus and not typically sensory, such as those from the amygdala and the hypothalamus, may have an important effect on spatial learning, the nature of which is not understood. Other studies (Wood et al., 1999, Hampson et al., 1999a) have found that spatial (and sensory dependent) information is often encoded by hippocampal units in combination with non-spatial (but also sensory) information. Whether there are cells that respond to a combination of sensory and non-sensory is to be seen.

Third, the results suggest that learning one or two watermazes requires similar amounts of tissue, a fact that has been discussed above. It has been suggested that the

temporal hippocampus place fields are bigger than septal hippocampus ones (Jung et al.,1994; but see Poucet et al., 1994). The size of the place field does not seem to have an important function in context differentiation as both rats with septal and temporal hippocampus seem to be equivalently good in this aspect of the task.

Fourth, in terms of processing capacity very little hippocampus is enough to support learning (Moser et al., 1995). The finding that rats with as little as 20% spared temporally or septally can learn a watermaze task satisfactorily support this. The reference memory task is in many ways a simple task for it involves learning the importance of one fixed position in space in a particular environment. In Chapter 6 evidence was presented that when the rat is required to use spatial information in a flexible way and favour different spatial positions on different days (delayed matching to place task) more hippocampal tissue was required. This suggests that learning about the spatial aspects of one environment is not all the hippocampus does, information about how the space is used is also laid down by this structure. Supporting this view Wood et al. (1999) and Hampson et al. (1999a) have found units in the hippocampus of rats that respond to physical aspects but also to behavioural aspects of the task. Accordingly and as discussed in the relevant point, learning one or two concurrent watermazes requires similar amounts of tissue.

A specific theory of hippocampal function in relation to the septotemporal axis is described by Lytton and Lipton (1999). They argue that the longitudinal axis acts as a rule of time in which the age of the memories is determined by their location along this axis with the most recent memories being closer to the most anterior end (temporal in rats). Strange et al.'s (1999) finding that presentation of novel pictures activates the anterior hippocampus while that of familiar pictures activates the posterior hippocampus could be considered to be a different interpretation of a similar pattern. Lepage et al. (1998) state that encoding of memories occurs in the anterior (temporal) hippocampus and retrieval in the posterior (septal) hippocampus. Memories that are encoded are, by definition more recent or novel than memories that are retrieved. Thus, Lepage et al.'s view maps well with the previous two studies. It is difficult to know how to match these theories with what is known about the anatomy of the posterior to anterior (equivalent to septotemporal) axis of the

human hippocampus. Although these are theories based on human research and a comparison is not straightforward, the findings presented in this chapter do not support these views.

The hypothesis that the hippocampus supports one memory process relies heavily on the fact that the intrinsic circuit is fairly homogeneous along the septotemporal but also on the fact that, although extrinsic information might be segregated in three areas along the septotemporal axis in DG, the intrinsic associational projections do not ensure the maintenance of this segregation. Computational theories of hippocampal function rely on the intrinsic circuit to explain hippocampus function. For example, the different elements of the circuit (EC, DG,CA3 and CA1) are attributed different functions in Hasselmo et al. (1996) and the particular importance of the CA3 associational system is highlighted by Treves and Rolls (1994), Rolls (1996) and Levy (1996). All of these models highlight aspects of the circuit that are homogeneous along the septotemporal axis. The results support these computational theories of hippocampal function that understand the intrinsic circuit as the basic architecture that determines the functional capacity of the hippocampus.

7.9 Conclusions

Rats with only temporal hippocampus spared can learn a reference memory task in the watermaze.

Rats with septal or temporal hippocampus spared differ in their responsiveness to aspects of the training protocol: number of trials per day and number of days of training respectively.

These results support the idea that the hippocampus is involved in one unique memory process. Whether this process supports one function or one set of related functions is a question that brings us back to the beginning of hippocampal research. How the segregation of extrinsic connections along the longitudinal axis and, conversely, the septotemporal extent of the intrinsic connections influence this function is, in my view, a fascinating question that remains to be answered.

Part 2

Hippocampal dependent memory consolidation

Chapter 8

Introduction to the questions on memory consolidation

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Chapter 8

Introduction to the questions on memory consolidation

8.1 General Introduction

The scope of the term consolidation, when used in relation to memory, is very broad. In this thesis the term will be used to refer to the process, beginning after acquisition of a particular memory, by which this memory becomes stable. This process can happen over a period lasting from days to years. This part of the thesis will focus on hippocampal dependent memory consolidation.

That such a process takes place and is dependent on the hippocampus is suggested by cases of human retrograde amnesia. It is often the case that damage to the hippocampus (often accompanied by damage to surrounding structures) results in the patient losing recently acquired memories but not those acquired long time ago. This is known as graded retrograde amnesia. Thus, it seems that over time memories become independent of the hippocampus and the process underlying this change is called hippocampal dependent memory consolidation.

Evidence for a process of consolidation from phenomena other than amnesias is scarce. Reminiscence or inverse forgetting, the process by which memory improves as time passes, has been suggested to be a natural consequence of consolidation. However, the conditions in which this phenomenon has been tested suggest that repeated testing might have been the reason for the improvement in memory (Keppel, 1984). For this reason, this chapter will focus exclusively on the evidence provided by the phenomenon of amnesia.

Cases of amnesia are, however, difficult to interpret. The first problem is the enormous variability between the data. This variability strives from different aspects of amnesia, most of which will be discussed in this chapter: 1. the localization and extent of the damage; 2. the type of information lost; 3. the presence of anterograde and/or retrograde amnesia; and, 4. whether the retrograde amnesia is flat or graded and, if graded, the length of the period covered by it. The second problem is that objective measures of memory are difficult to obtain and pre-traumatic assessment is not always available.

This chapter means to introduce the notion of consolidation and its pitfalls, not to review the amnesia literature, therefore, reference to the human cases of amnesia is limited to what is necessary to illustrate a particular point. Consolidation research addresses several, often orthogonal, issues which makes it difficult to subdivide the subject in sections. However, with the aim of highlighting the current issues concerning the subject and the most urgent questions, I have divided this introduction in three subheadings: what, where and when, in an attempt to clarify what it is, where it takes place, and for how long. The chapter will end with a review of the few animal experiments addressing the issue of consolidation.

As where and when this process takes place is directly related to its nature, I will begin by describing these two points.

8.2 Where

Two broad areas in the brain have long since been associated with the memory processes that are affected in amnesia: the diencephalon and the mediotemporal lobe. The former was first identified as relevant by Gudden (1896) in a neuropathological study of Korsakoff's syndrome patients. The mediotemporal lobe was identified after a study of the brain of a patient who suffered from severe retrograde amnesia (von Bechterew, 1900). Since then we talk about diencephalic and mediotemporal amnesia.

As the focus is on hippocampal dependent consolidation, diencephalic amnesia will not be discussed any further.

8.2.1 Mediotemporal amnesia.

It can be observed after viral encephalitis (Rose and Symonds, 1960), posterior cerebral artery occlusion (Benson et al., 1974), ischemic injury (Volpe and Hirst, 1983; Zola-Morgan et al., 1986) and temporal lobe removal for the treatment of, otherwise, intractable epilepsy (Scoville and Milner, 1957; Penfield and Milner, 1958).

Mediotemporal amnesic patients are friendly, show a sense of humour and have insight into their condition.

The patient H.M. was key in the understanding of this type of amnesia. H.M., who suffered from intractable epilepsy, received surgery aimed to remove the areas source of the epilepsy. The temporal lobe, including the entorhinal, perirhinal and periamygdaloid cortices, the parahippocampal region, the amygdala, hippocampal gyrus, the anterior 2/3 of the hippocampus and the uncus were bilaterally removed (Scoville and Milner, 1957). H.M.'s epilepsy was cured but he became amnesic both anterogradely and retrogradely (Corkin, 1984). The latter was graded (Corkin 1965, Milner 1965, 1968).

Another example is that of patient R.B. (Zola-Morgan et al., 1986), who suffered an ischemic event during an open-heart operation which resulted in damage restricted to CA1. He died 5 years later. During that time he had normal intellectual capacities with the exception of anterograde amnesia. He had no signs of retrograde amnesia, but the tests used would not have detected memory deficits for the year or two previous to the injury (Squire, 1989).

DRB, another amnesic patient whose amnesia was caused by encephalitis (Damasio et al., 1985), suffers both anterograde and retrograde amnesia, the latter complete. His damage affects the whole of the temporal cortex including memory associated

structures and anterolateral areas, but also the orbitofrotal cortex and the basal forebrain.

These and other cases illustrate the variability in cause, physical extent and severity of the damage in mediotemporal amnesia. However, although it has been suggested that the severity of the resulting amnesia is positively correlated with the extent of the damage (Milner, 1974), damage to the hippocampus is a common feature in all these cases. As a consequence the hippocampus is understood as a key structure in consolidation.

Within the temporal lobe it was believed for some time that damage to the amygdala was partly responsible for amnesia. Studies in monkeys revealed that lesions to the hippocampus (Zola-Morgan and Squire, 1986) did not generate amnesias as severe as those that also included the amygdala (Mishkin, 1978). However, later studies revealed that this differential severity was a result of extra-hippocampal and non-amygdalar damage associated with hippocampus plus amygdala lesions but not with hippocampal lesions alone. A lesion to the amygdala only had no effect on memory (Murray and Mishkin, 1986; Zola-Morgan et al., 1989b).

The remainder of this chapter will focus on the particular involvement of the hippocampus in memory consolidation.

Damage to the hippocampus is very often partial in cases of amnesia. This is a fact that is generally ignored in the literature. Very often particular cases are discussed on the grounds of complete absence of hippocampal function, even though it is acknowledged that part of the hippocampus is spared (Vargha-Kahdem et al., 1997; Reed and Squire, 1998). In my view this is a key issue on the variability observed across cases of amnesia and, for this reason, it is further explored in Chapter 10. Its relevance is highlighted when one considers that models of memory consolidation (discussed below) suggest interactions between the neocortex and the hippocampus as the base for the process. If part of the hippocampus is spared, these interactions could still take place and, either support a continuing consolidation process or facilitate retrieval.

It is believed that the bigger the extent of the damage, the more severe is the associated retrograde amnesia both in terms of types of memory affected and temporal extent of the memory loss (Milner, 1974). Theories of hippocampal function that, in one way or another, suggest a correlation between the age of the memory and the position along the hippocampal longitudinal axis (discussed in Chapter 7; Lepage et al., 1998; Strange et al., 1999; Lytton and Lipton, 1999) acquire a different, and more interesting, light in these circumstances.

Thus, concluding this section, amnesias results from damage that can be, although this is not always the case, limited to the hippocampus. When the damage is limited to the hippocampus, the extent is also variable.

8.3 When

Retrograde amnesia associated with permanent temporal lobe damage can, sometimes, cover the whole life of the patient (e.g. Cermak and O'Connor, 1983; Damasio et al. 1985; Warrington and Duchen, 1992). Other times it is very minor, if present, like in the case of patient R.B. (Zola-Morgan et al., 1986). Retrograde amnesia tends to cover a larger period for autobiographical memories than semantic knowledge (e.g. Cermak and O'Connor, 1983; Vargha-Khadem et al., 1997). A flat gradient of retrograde amnesia (old and recent memories are equally affected) is generally associated with damage extending beyond the temporal lobe (e.g. Cermak and O'Connor, 1983).

Assessing memory for remote events is not easy, especially when these are autobiographic. Kapur et al. (1999), for example, report a patient with damage to CA1 and CA3 and complete loss of autobiographical memory for the previous 20 years. The patient, however, declares being familiar with the memories.

The same trauma can result in retrograde amnesia of different severity. For example, Squire et al. (1975) report up to 3 years of retrograde amnesia after electroconvulsive therapy (ECT), while Goldberg and Barrett (1985) report decades of amnesia.

Retrograde amnesia is sometimes cured if the trauma is temporary (Barbizet et al., 1970) and has been reported to recede even after permanent damage (the case of H.M.), although this could reflect a difference in the assessment method rather than a real development in memory.

The main point, however, is that the period of graded retrograde amnesia can cover several decades of the patients life. According to the theory, that must be the length of the consolidation process. The question is, how is it possible for a physiological process to take such a long time?

Retrograde amnesia is often accompanied by an impairment in the acquisition of new memories (anterograde amnesia). This highlights the, may be obvious but nonetheless, important fact that the same structure on which memory consolidation is dependent plays an essential role in memory acquisition. Whether the hippocampus is involved in consolidating memories that can be acquired without the hippocampus is another question.

Relationships between the severity of retrograde and anterograde amnesia have been reported before (cf. Squire and Alvarez, 1995; Nadel and Moscovitch, 1997; but see Shimamura and Squire, 1986). Nonetheless, they can appear independent of each other (Wood et al., 1982); for example anterograde amnesia can appear with no sign of retrograde amnesia (Cohen and Squire, 1981; Winocur et al.,1984) and vice versa (Kapur, 1993). Also, one can recede leaving the other in place (Goldberg et al., 1982). This variability in the data is likely to be entirely the result of variability in the structures affected and the type of trauma, but it also suggests that the mechanisms underlying the two types of amnesia could be independent.

An alternative to the theory of memory consolidation is the argument that what we believe to be retrograde amnesia might be anterograde amnesia. The onset of amnesia might be gradual and start much earlier than believed, i.e. at around the period in the patient's life where retrograde amnesia begins. According to this theory retrograde amnesia is never real retrograde amnesia but rather the beginning of the anterograde amnesia. In the case of epileptic patients, for example, it can be argued that the

seizures themselves might have started earlier than believed and cause anterograde amnesia. Although this is possible it does not explain the numerous situations in which accounts of normal premorbid memory exist.

8.4 What

Two relevant aspects of amnesia, what type of memory is lost and why, influence how the process of consolidation is understood and its nature explained. In this section a brief discussion on each of these aspects is followed by a description of the implication of a theory of memory consolidation and three key models of the process.

8.4.1 What type of memory is affected?

Amnesic patients are physically normal and have intact intellectual capacities (normal IQs). They also display normal short term (e.g. Kapur et al., 1997) and procedural memory (memory for skills; Schneider,1912; Cohen and Squire, 1980; although see Mishkin et al.; 1984).

Amnesia affects declarative memory, both semantic and episodic subdivisions. Episodic and semantic memory are not always equally affected in amnesia. The latter is usually less impaired if at all (Warrington, 1975; Kinsbourne, 1987; Vargha-Kahdem et al., 1997; but see Shimamura and Squire, 1986 and Squire and Zola-Morgan, 1988; cf. Nadel and Moscovitch, 1997), suggesting that the mechanisms underlying these two types of memories might be of different nature. Kinsbourne (1987) argues that semantic memory is affected as a consequence of the loss of episodic memory. The necessity to 're-experience' the event (episodic memory) in order to retrieve the semantic memory that was acquired through it, makes the latter unattainable in the absence of the former. This is one example of the close relationship between retrieval and consolidation deficits in amnesia studies, a subject that will be discussed further down.

8.4.2 Alternative theories

According to the theory what determines whether a memory is preserved after the onset of amnesia is its age. Thus, time is the main determinant of the completion of this process. However, it is also the source of one of the main problems surrounding the theory. I am reluctant to accept that such a process could take years to complete. It is difficult to imagine such a lengthy physiological process and thus one cannot help but believe that some kind of psychological 'putting the memories behind' type of process must also affect the outcome of amnesia. Most alternative theories play around the problem of time by trying to explain the data in terms other than memory age. Bartlett (1932), for example, argues that a consolidation process is not necessarily the best interpretation for the phenomenon of graded retrograde amnesia. While recent memories are numerous, not necessarily relevant, and rich in detail, remote memories are generally salient but scarce in details. It is possible that amnesia results in the loss of irrelevant information and non-salient detail. This would result in remote memories being practically untouched and recent memories being drastically filtered, a pattern that could look like graded retrograde amnesia. Neisser (1984) suggests that older memories are fewer and thus do not compete with others at the time of retrieval. As amnesics are very sensitive to interference during retrieval (Warrigton and Weiskrantz, 1970, 1978), older memories are differentially easier to retrieve. Glenberg and Swanson (1986) also suggest that older memories are more distinct because they are fewer. McGeogh (1942), in a more psychological account for the process, argues that older memories are, in content and context, more distinct from the present and, therefore, not as likely to interfere with present cues as recent memories.

None of the above, however, explains why certain retrograde amnesias cover only a few years and others cover decades. When the amnesia covers only a few years it is specially difficult to use the arguments outlined above, because why should memories acquired, for example, 3 years before the trauma be better filtered by forgetting or less subject to interference than memories acquired 2 years before?

Moreover, Squire and Cohen (1979) addressed the question raised by Barlett (1932), namely that amnesia affects only non-salient irrelevant information, and found that the retrograde loss of memory affected salient and non-salient information in the same degree.

Thus these type of interference theories fail to give a reliable account of the data.

8.4.3 Implications of the theory of memory consolidation

The theory of hippocampal dependent memory consolidation suggest a time-dependent process by which memories become independent of the hippocampus.

There are various important implications of this theory that are likely to influence the way the nature of the process is understood. Some of these implications are compulsory, others are mere possibilities.

There are two necessary consequences of the theory. First, in order for memories to become independent of the hippocampus, their retrieval has to become independent of the structure. Second, in order for memories to be retrieved without the hippocampus, they need to be stored somewhere else. The neocortex is generally understood as the brain area that serves ultimately as the storage of memory.

These implications are important because, although consolidation is generally understood as a strengthening process by which memory traces become stable and less vulnerable to interference, in order to explain the data, this concept needs to be broadened to include retrieval and storage. Thus, consolidation needs to refer to a process by which memories become independent of the hippocampus for their retrieval and storage.

Once the concept is broadened another implication follows. The process by which memory traces are strengthened over time, does not necessarily require the hippocampus. It is possible that this process takes place in the neocortex, the ultimate memory repository, and that the hippocampus merely supports memory retrieval, and

possibly also storage, while the process takes place. In fact, amnesias do not give evidence for an involvement of the hippocampus in anything but retrieval, as retrieval is the only measure of memory available at the moment.

The possibility that the problem of amnesia is one of retrieval is highlighted by the fact that memories are not always completely lost in amnesia. Retrograde amnesia is more severe for spontaneous remembering than for cued responses (Tulving, 1983) a phenomenon which, although potentially different in nature, has been more widely studied in anterograde amnesia (priming; Weiskrantz and Warrington, 1970). Sagar et al. (1985), for example, find that patients can remember episodic events if heavily cued. This phenomenon suggests that memory was not lost, but merely out of reach.

It is also possible, however, that the hippocampus plays a role during consolidation other than mediating retrieval. In fact, models of memory consolidation are based on this possibility and suggest interactions between the neocortex and the hippocampus as the base of the process.

8.4.4 Some theories on the nature of consolidation

Most modern theories of memory consolidation suggest that the nature of consolidation takes the form of generation of memory traces through re activation of the cortico-hippocampal connections. These theories understand memories as composed of elements that are distributed across the neocortex according to their nature. That neocortical damage results in theme-specific amnesias, further supports this idea. The hippocampus acts as link between the various elements that compose a memory. Squire and Zola-Morgan (1988), for example, suggest that rehearsal or repetition might generate several copies of a memory: 'additional representations of the original experience are established'. 'As this process of reorganization or consolidation continues, representations gradually become independent of the structures damaged in amnesia'. This idea is further explored by Squire and Alvarez (1995) who propose that reactivation of a particular memory results in activation of the neocortical-hippocampal traces that link the different components of the memory.

This activation results in strengthening of cortico-cortical connections, which eventually become strong enough that activation of a fragment of the memory result in reactivation of the whole memory through these cortico-cortical connections in a hippocampal independent manner.

Nadel and Moscovitch (1997) propose a somewhat similar model in which every time a memory is reactivated a new cortico-hippocampal trace is formed. In their 'multiple trace theory', consolidation consists on new memory traces being created through reactivation and established in a distributed manner along the hippocampus. As time passes the number of reactivations might increase and, with it, the number of hippocampal traces. A lesion to the hippocampus is then less likely to affect the memory for, unless the damage is complete, some traces are likely to have survived. This proposal would account for two important aspects of amnesia. First, generally, the bigger the extent of the damage, the more severe retrograde amnesia is (cf. Moscovitch and Nadel, 1998). Second, damage to the hippocampus is not always complete. According to the model, complete damage would result in a flat gradient of retrograde amnesia. Nadel and Moscovitch (1997) propose that this should be the case for episodic memories, which they do not believe to consolidate, in the sense of becoming independent of the hippocampus.

Murre (1996) presents a model, TraceLink, by which, also as a result of repetitive activation, connections are strengthened within the structure that stores the memory (neocortex) while the connections between this and the structure that acts as a link (hippocampus) disappear. Damage to the link will only erase those memories that are still dependent on it. The hippocampus 'acts as a temporary "scaffold" for new memories, serving as an intermediate "link station" of connections, before they are well established at a cortico-cortical level' (Murre, 1996). When remembering an episode some elements of the event will be retrieved thanks to connections with other elements of the event. These connections must occur over long ranges of the neocortex but, because of the sparse and modular connectivity of the neocortex (Murre and Sturdy, 1995) these connections do not tend to occur for newly learned representations. The hippocampus is responsible for their establishment.

All of these theories propose memory reactivation as the trigger of the process of consolidation. They do not explain however, what triggers reactivation. Is it a spontaneous and internal process? Or is it driven by external stimuli? If the former, what is the use of a process by which memories are spontaneously reactivated over the period of years? If the latter, only memories whose content is relevant in present situations will be reactivated. However, in amnesia, a very relevant recent memory, likely to have been reactivated several times, such as, for example, the route taken to work every day for the previous 3 years, can be lost.

8.4.5 Consolidation: time- or content-dependent process?

All of these theories put forward reactivation as the mechanism for consolidation. It is, therefore, implied in these theories that those memories that are reactivated are more likely to be spared after amnesia. This, in my view, is very different from the original notion of a time-dependent process by which time is the main parameter determining whether a memory is spared or not in amnesia.

Abandoning the original idea of a time-dependent consolidation presents several advantages. The first reason is that there is no longer any need to explain how it is possible that the process can take as long as years to complete. The loss of memories acquired years before the trauma can now be attributed to a lack of reactivation of these memories during those years. There are however many cases of amnesia where a very defined period of the patients life, but not events previous to that point, is lost to memory. Theories like the ones described above cannot account for this type of amnesia and time must be considered here as the principal parameter.

The second reason is that, without the notion of a time-dependent passive process, the nature of hippocampal function can be more readily incorporated into theories attempting to explain retrograde amnesia. This is because consolidation is no longer understood as a passive process occurring within the hippocampus but, rather, as an active mechanism triggered by present events and involving both the hippocampus and neocortical structures. This should result in consolidation being susceptible to external manipulation, which is essential for any useful experimental design. In

relation to the nature of the role of the hippocampus in memory, its critical role in episodic memory becomes the main criticism against the notion of a slow time-dependent consolidation process. Episodic memory, in order to be accurate, requires a fast consolidation system. And yet, it is episodic memories that are more severely affected by hippocampal damage, suggesting that, despite a possible fast consolidation mechanism they do not become independent of the hippocampus. Is this because their retrieval is permanently dependent on the structure?

The third reason to abandon time-dependence is the impossibility to distinguish between retrieval and consolidation deficit in amnesias. An issue that is highlighted by the importance that theories of hippocampal-dependent consolidation place on reactivation. Memories are not defined entities that exist in an 'all or none' fashion and, therefore, it could be possible to retrieve the same memory (or different aspects of it) through completely different physiological mechanisms. It is possible that this is what occurs during the phenomenon of priming: the memory is being retrieved but, is it similar in nature to the memory that would have been retrieved spontaneously, had the hippocampus been spared? The hippocampus, when intact, might play a fundamental role in spontaneous recall by means of its connections, through the entorhinal cortex, with neocortical structures of different modalitites. It might serve as the link between the different components of a memory distributed across the neocortex according to their modality. When the hippocampus is dysfunctional a memory might be primed by direct reactivation of the memory trace. Whether this memory is as complete as that retrieved via the hippocampus would depend on the nature of the cortico-cortical connections associated with that memory, but it is possible that only the component of the memory that is directly being activated is retrieved.

8.5 Animal experiments

Most key questions on memory consolidation arise from the variability in the data obtained from human amnesias. In this situation, animal experiments become an

essential tool of the study of memory consolidation, as they provide the means to control for the effect of different parameters on the results.

Animal experiments on consolidation are scarce. A selection of them, across species and tasks, are described here in some detail.

Zola-Morgan and Squire (1990) trained monkeys in 5 successive visual discriminations, each of which involved 20 pairs of stimuli and lasted 10 days. Discrimination sessions were separated by 4 weeks. Two weeks after the end of the last visual discrimination the monkeys received lesions affecting the hippocampus, the subiculum and the entorhinal cortex. Testing took place 2 weeks later. The results were as follows: operated monkeys performed significantly poorer than controls during the testing of discriminations learnt 2 or 4 weeks before surgery, but were similar to controls in discriminations learnt more than 4 weeks before surgery. Two points need highlighting. First, operated monkeys are not at chance even for discriminations learnt soon before surgery. Second, although the performance of operated monkeys is better for discriminations learnt 16 weeks before surgery than for those learnt 2 weeks before (monotonic function), forgetting in control monkeys is steeper. This suggests that the similarity across groups in performance for remote discriminations could be better explained by forgetting in control monkeys than by a graded retrograde amnesia in operated monkeys. Thus, although faint evidence of graded retrograde amnesia is found, the lack of a complete amnesia for recent memories (maybe due to the first testing interval being already too long) and steep forgetting in controls, constitute down sides to this experiment.

Wiig et al. (1996) performed a similar within subject experiment in rats with fornix lesions and found graded retrograde amnesia for visual discrimination covering 4 to 6 weeks prior to surgery.

Winocur (1990) performed a between subject experiment on memory for socially transmitted food preference. This is a natural phenomenon by which a rat (called here the observer) will tend to chose to eat food, whose smell it has previously detected in the breath of another rat (called here the demonstrator). The advantage of expressing

this behaviour is that if the demonstrator rat is alive, the food it ate must have been safe. This paradigm was first used by Galef and Wigmore (1983). It is known to involve the association between two odours: carbon disulfide, which is a constituent of the demonstrator's breath, and the odour of the food that this rat has just ingested (Galef et al., 1988). Either of the two odours alone would not generate the characteristic preferential food choice by the observer but the presentation of both odours (without the actual demonstrator rat) is enough.

An experimental rat was coupled for 30 minutes with a demonstrator rat which, previously, had been given food with a particular smell. Lesions to the dorsal hippocampus or sham lesions were made immediately after presentation or 2, 5 or 10 days later. Testing took place 10 days after surgery. Memory was measured as the percentage of diet with familiar smell, versus diet with unfamiliar smell, eaten. Lesioned rats were at chance for presentations made immediately or 2 days before surgery but they were as good as controls for memory of presentations made 5 or 10 days before surgery. Lesioned rats displayed a clear gradient of retrograde amnesia. Sham animals revealed forgetting over time. According to this experiment hippocampal dependent consolidation of socially transmitted food preference requires 2 to 5 days to complete.

In 1992, Kim and Fanselow, measured hippocampal dependent consolidation of contextual and tone fear conditioning in rats. They used a between subjects design by which rats were given electrolytic lesions affecting the dorsal hippocampal (but see below) either 1, 7, 14 or 18 days after training. Testing took place 7 days after surgery. While all the lesioned animals displayed normal tone fear conditioning regardless of the time interval, contextual fear conditioning was not present in rats trained 1 day before surgery and was impaired, with respect to controls, in rats trained 7 days before surgery. Again, a gradient of retrograde amnesia is found in hippocampal lesioned rats. A degree of forgetting is also observed in shams. The interesting point made by this study is that not all types of declarative memory, and even more, not all types of fear conditioning, are consolidated within the hippocampus. Tone fear conditioning, for example, is independent of this structure.

A series of experiments have been described and evidence of graded retrograde amnesia is found across various tasks. Similar experiments performed with rats in the watermaze, however, invariably result in flat gradients of retrograde amnesia for platform positions learnt up to 14 weeks before surgery (Bolhuis et al., 1994; Mumby et al., 1999a; and numerous Soc. Neurosci. Abstr., cf. Nadel and Moscovitch, 1997).

Is a flat gradient characteristic of all spatial tasks? Ramos (1998) reports a gradient of retrograde amnesia covering up to 64 days for spatial information in a radial maze in rats with electrolytic lesion to the dorsal hippocampus. Memory, however, was measured as performance over 18 trials. It is difficult, thus, to conclude whether the above chance performance (only just) observed on animals lesioned 64 days after training is a result of memory or re-learning. In any case, it is a time-dependent effect. Cho et al. (1993, 1995) find that lesions to the hippocampus or entorhinal cortex also result in graded retrograde amnesia for spatial information in a radial maze. Gaffan (1993), however, finds a flat gradient of amnesia for scene discrimination in monkeys with fornix lesions. So, although flat gradients are not exclusive to the watermaze, they are not necessarily observed in all spatial tasks.

Animal experiments described here indicate that human graded retrograde amnesia can be modelled but they do not give any insight, to date, on the mechanisms underlying consolidation. Key questions highlighted in previous points remain unanswered. For example, the relationship between retrieval and consolidation deficits is not addressed. Some studies involve re-learning, which should facilitate retrieval. However, this measure could well reflect new learning instead.

These studies do, however, clarify a point in relation to theories of hippocampal function. In none of these experiments are memories behaviourally reactivated after training and yet a graded amnesia is found in many of them. In order for these experiments to fit the models, one would have to argue that memories are spontaneously reactivated during the days or weeks that follow acquisition. This is true regardless of whether the memory is acquired in one unique trial (Kim and Fanselow, 1992) or several trials (Wiig et al., 1996).

These experiment also suggest that the length of the process of consolidation varies with the task at hand.

8.6 Conclusions and questions

Hippocampal dependent memory consolidation is understood as the process by which memories become independent of the hippocampus with time. This independence, in order to explain the human and animal literature, needs to extend to the process of retrieval.

The aim of this chapter was, partly, to highlight certain still unanswered questions that are key to the understanding of memory consolidation. One such question relates to the relationship between consolidation and retrieval. The differential expression of old versus recent memories in retrograde amnesia reflects a differential capacity of the patient to retrieve those memories. This retrieval capacity, however, might be independent of a consolidation process.

Another question is raised by models of memory consolidation, where hippocampal mediated reactivation of neocortical memory traces is proposed as the mechanism underlying this process. If the hippocampus is responsible for this reactivation-dependent process, it might be possible to preserve memories by artificially reactivating them in the absence of the hippocampus. This might also help routes of retrieval that are impoverished by hippocampal damage.

Finally, a very important issue in memory consolidation and one that is often ignored, is the possibility that the temporal and qualitative extent of the memory loss depends on whether hippocampal damage is complete or partial.

Chapter 9

Temporary inactivation of the hippocampus reveals a role in acquisition, consolidation and retrieval of memory

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Chapter 9

Temporary inactivation of the hippocampus reveals a role in acquisition, consolidation and retrieval of memory

9.1 Introduction

Acquisition, consolidation and retrieval have been identified as three distinctive stages in memory processing. Storage and forgetting could also be considered to be memory stages, however, as they fall beyond the scope of this chapter, little will be said about them.

Traditionally, the involvement of a particular structure in any cognitive process is studied by exploring the effect that lesioning the structure has in a chosen behavioural paradigm. In Chapters 1 and 8 we have seen numerous examples of this type of approach in the study of hippocampal dependent memory processes. The involvement of the hippocampus in acquisition of several memory tasks has been established this way (Morris et al., 1982; Jarrard, 1995; Bunsey and Eichenbaum, 1996). However, permanent lesions affect all memory stages subsequent to the lesion, a problem that limits the use of this technique in studies attempting to identify the differential involvement of the hippocampus in the various memory stages. Only a lesion made before retrieval spares the other stages (acquisition and consolidation) but, even in this case, the possibility of comparing any impairment in retrieval with the capacity for new learning is lost.

This problem is particularly apparent in the study of memory consolidation. In amnesias as well as in the animal experiments on consolidation presented in the

previous chapter, it cannot be determined whether the memory deficit is due to the incompleteness of an internal stabilising process of consolidation or an incapacity to retrieve the memory. Or is consolidation considered complete exclusively when retrieval becomes independent from the hippocampus? To address this issue, one needs to study the process of consolidation and retrieval independently, however, inactivation of consolidation without affecting the capacity for retrieval, has not been possible to date.

The use of a new compound, LY326325, allows reversible neural inactivation and therefore permits switching off the hippocampus during specific memory stages. Other such compounds have been used before, however, they required solvents, such as DMSO, that when injected into the brain had harmful effects on the tissue. LY32625 (LY) has the advantage of being soluble in aCSF. As this is an innocuous solvent, any impairments found can be attributed to the LY-driven temporary block of glutamate receptor subunits GluR1-5.

The aim of the experiments presented in this chapter was to disclose the possible differential involvement of the hippocampus in the different memory processes (acquisition, consolidation and retrieval) required in a traditional spatial reference memory task in the watermaze. This was done by temporarily inactivating the hippocampus with LY326325.

I did not contribute, in terms of labour, to all the work presented in this chapter. I did, however, contribute during the general discussions where decisions about the necessity of the different experiments and their protocol were made. As all the aspects are relevant for the general picture, I have included a complete description of all the experiments that form part of it. This work is published in Riedel et al. (1999).

I have organized the chapter in three experimental sections and a final general discussion. The experimental sections are: technical controls, behavioural experiments and behavioural controls. Each of them is structured in methods, results and discussion. The methods section is, sometimes, embedded within the results. A more complete account of the methodology can be found in the General Methods chapter.

What follows is a small introduction to each of the experimental sections.

9.1.1 Technical controls

Because the use of LY326325 is new in behavioural research in general and in hippocampal research in particular, a series of technical controls were required to establish the efficacy of this compound to inactivate fast transmission and to do so only in the hippocampus.

In traditional lesion studies the effect of the damage is assessed at the end of the experiment through within subject histological analysis. However, the same feature that constitutes the advantage of temporary inactivations, i.e. the lack of residual effect once the drug is washed out, demands parallel between subject controls to assess the efficacy of the drug.

First, to establish that LY infused into the hippocampus temporarily inactivates fast transmission, an acute electrophysiological control was made. Second, in most of the behavioural experiments that constituted this study the hippocampus was inactivated for 7 days by means of an implanted osmotic micropump. Therefore, it was necessary to ensure that chronic infusion of LY in this manner successfully inactivated the hippocampus during the period of time the pump was claimed to be active. Chronic electrophysiological controls were made to ensure this was the case. And finally, to establish whether LY is restricted to the hippocampus and where within the structure, the spatial distribution of differential brain activity in aCSF and LY rats was established. This was done by taking measures of cerebral glucose use by [^{14}C]2-deoxyglucose autoradiography (2-DG).

9.1.2 Behavioural experiments

These constitute the core of this study. One watermaze protocol was used in all these experiments as a scaffold. The time of inactivation was varied such that this occurred either through acquisition and/or retrieval or during what is called the consolidation period (time between training –acquisition- and testing –retrieval-).

9.1.3 Behavioural controls

A series of controls were made to establish whether the results obtained in the behavioural experiments reflected the involvement of the hippocampus in the different memory processes studied or were the result of other, uncontrolled for, experimental variables. Task, structure, treatment-time and memory-process specificity of the results was explored.

9.2 Technical controls

I did not contribute to any of the three experiments described in this section.

9.2.1 Methods and Results

9.2.1.1 Temporary inactivation of fast glutamatergic transmission by acute LY infusion

Carried out by Holly Bridge, Steve Martin and Beatrix Poeschel. See Methods, p. 27 and 40.

The effects of acute infusion of LY were measured by in vivo electrophysiology, to establish that LY temporarily inactivates fast glutamatergic transmission in both the dentate gyrus and CA1 regions of the hippocampus.

Rats, under urethane anaesthesia, were infused with either aCSF or LY into the dorsal hippocampus. Field potentials were measured in dorsal DG (6 aCSF and 6 LY treated rats) or CA1 (8 aCSF and 8 LY) upon stimulation of the perforant path. Acute infusion of LY, but not aCSF, successfully inactivated fast glutamatergic transmission in the DG and CA1 regions of the dorsal hippocampus (Fig. 9.1.a) as revealed by 90% decrease in field potential slope after the infusion. The maximum decrease was observed at around half an hour of the LY infusion. After 6 hours the slope was 20 to 25% of the original slope in DG and 50% in CA1.

The capacity of LY to considerably inactivate fast glutamatergic transmission in the hippocampus after acute infusion for at least 6 hours was established.

9.2.1.2 Inactivation time-course resulting from chronic LY infusion

Carried out by Eva V. L. Roloff, Beatrix Poeschel and Gernot Riedel. See Methods, ps. 27 and 40.

In vivo electrophysiology of freely moving rats implanted with aCSF or LY micropumps was carried out to establish the time-course of LY inactivation of fast glutamatergic transmission in this structure.

After stimulation of the perforant path, field potentials were recorded daily in the dorsal DG of freely moving rats before, during and after chronic infusion of LY or aCSF in the hippocampus by means of an implanted micropump. Baseline measurements were taken when all the rats were under aCSF chronic infusion. Micropumps were then changed for new ones containing either LY or aCSF. This caused a striking decrease in field potential slope in the LY treated rats but not in the

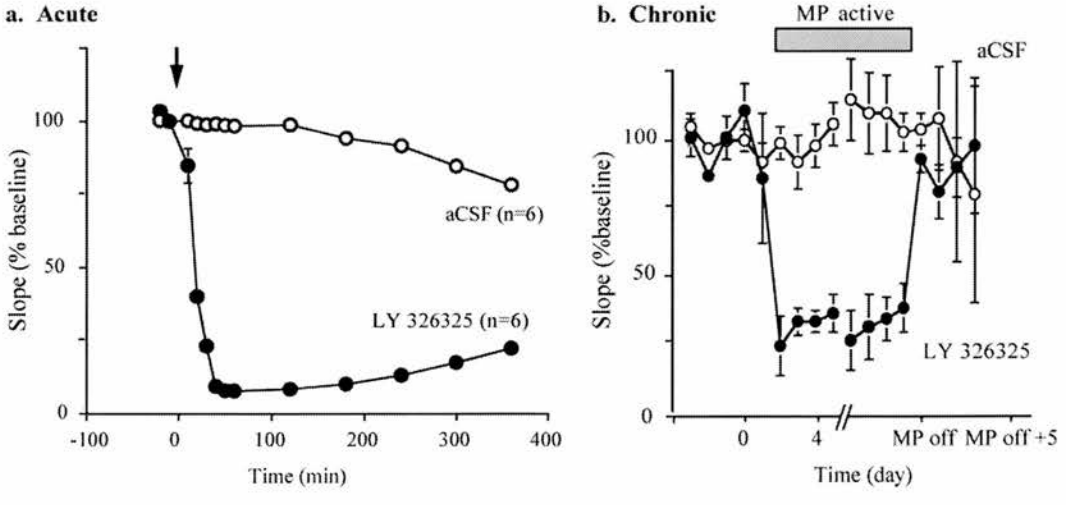

Figure 9.1: (a) Decrease in DG and CA1 glutamatergic transmission slope after acute (arrow) infusion of LY as compared with aCSF treated animals. **(b)** Decrease in DG glutamatergic transmission slope and spike after chronic infusion of LY. Split plot locked both forward (time when micropump was changed) and backward (field-potential restored to within 20 % of normal). (± S.E.M.). MP: micropump.

aCSF group as illustrated in Figure 9.1.b. This decrease lasted for 9 ±1 days. The aCSF treated rats had normal field potentials during that time. Recordings made after the micropumps had become inactive showed that field potentials recovered normal appearance. This experiment revealed that fast glutamatergic transmission is strikingly and quickly inactivated in rats implanted with LY micropumps and that this inactivation was maintained for 9±1 days. Normal responses were restored abruptly after this time.

9.2.1.3 Spatial distribution of inactivation resulting from chronic LY infusion

Carried out by Amy G.M. Lam, James McCulloch, Gernot Riedel and Richard G.M. Morris. See Methods, p. 41.

Rats were implanted with aCSf or LY micropumps. Four days (micropump active) or 11 days (micropump exhausted) later rats were sacrifized and local cerebral glucose utilization was measured with [^{14}C]2-deoxyglucose autoradiography (2-DG). It was revealed that LY resulted in decreased glucose use mainly in the dorsal hippocampus

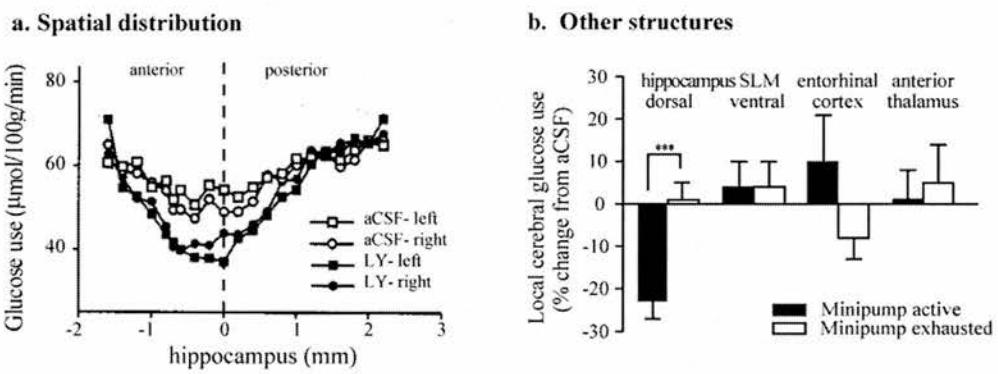

Figure 9.2: (a) Decrease in Glucose utilization during LY infusion along the dorsal hippocampus. The dashed line represents anterio-posterior cannula position. **(b)** LY driven change in glucose use relative to aCSF treated subjects in different brain structures during and after micropump infusion. SLM: stratum lacunosum moleculare.

(Fig. 9.2.a) but that it did not affect other memory related structures (numerous brain structures were analyzed) such as the thalamus (Fig. 9.2.b). A non-significant compensatory effect was observed in EC.

9.2.2 Interim Discussion

The above experiments demonstrated the functional, temporal and spatial efficacy of the infusion method.

It was established that LY326325 successfully inactivates fast transmission in the hippocampus as demonstrated by the massive decrease in slope (% of baseline) in both DG and CA1.

It was also established that, when caused by micropump implantation, this inactivation had a rapid onset, lasted several days (9 ± 1) and recovered abruptly. The aCSF condition revealed that aCSF, on the other hand, had no effect on hippocampal activity. The abrupt recovery of function is an important asset of the technique as it assures that the area of hippocampus affected by LY is generally either switched off or fully active. If inactivation was partial for any length of time it would be difficult to conclude whether any impairment found in behaviour was a result of the inactivation itself or of the noise that is surely associated with partial inactivation.

It was also established that inactivation is restricted to the hippocampus, not affecting other memory structures, and limited to the dorsal part of the structure. 2-DG technique revealed that cerebral glucose use on day 4 of implantation was reduced by 23% in an area covering approximately 1.5 mm anterior and 1.5 mm posterior to the cannula placement. The placement position was fairly homogeneous across animals and, as described in the general methods, was aimed at the dorsal hippocampus 4.5 mm posterior from Bregma. As the total length of an extended hippocampus is approximately 8 mm, between 35 and 40% of the longitudinal axis of the hippocampus is inactivated and that this region is centred in the septal 2/3 of this axis. In part 1 of this thesis it was concluded that when training is given at a very high trials/day ratio during less than 6 days, the temporal hippocampus alone cannot

support learning as measured in a transfer test. Considering the protocol used during this behavioural experiments (10 trials/day for 4 days) it seems unlikely that the ventral hippocampus, left intact with this technique, is capable of supporting any spatial learning during the 4 days of training. Also, unlike what is true for partial hippocampal lesions, inactivation of fast transmission in part of the hippocampus is likely to cause noise in the rest of the structure rendering it inefficient. These two arguments support the idea that inactivating the septal hippocampus is likely to have the effect of inactivating the whole of the structure.

9.3 Behavioural experiments

Here experiments aiming to disclose the involvement of the hippocampus in acquisition, consolidation and retrieval are described. Those experiments focused on acquisition and retrieval are designed in such a way that their protocols support each other. For this reason they are presented together.

9.3.1 Methods

9.3.1.1 Acquisition/retrieval

Run by Eva Roloff, Jacques Micheau and myself and rats implanted by Gernot Riedel. See Methods, ps. 27 and 32.

Micropumps (aCSF and LY) were implanted 7 days before start of pre-training and disconnected the day before the last day of training. See Figure 9.3. The reason to disconnect the micropumps then and not after the end of training resides in the findings described in section 9.2.1.2 to the effect that, after disconnection of the micropump normal responses are restored over the period of 24 hours. Thus, disconnecting the micropumps the day before the last day of training ensures that the inactivation does not affect any part of the period of consolidation and suggests that the last day of training is still under the influence of LY. Half an hour before testing,

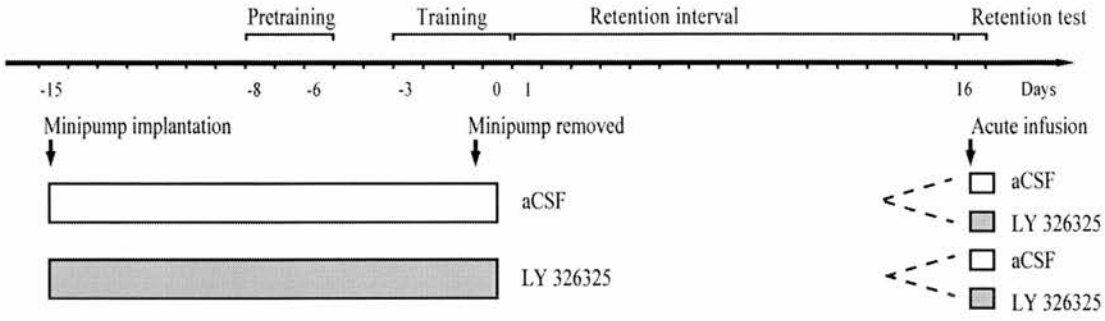

Figure 9.3: Schematic drawing of the experimental protocol used in the acquisition/retrieval experiment.

16 days after the end of training, aCSF or LY was infused acutely into the hippocampus through cannulae, implanted at the same time as the micropumps.

Pretraining consisted of 3 days of non-spatial training in the downstairs pool with the Atlantic platform. Training, starting 2 days after pre-training, was given also in the downstairs pool. It consisted of a spatial reference memory task with the Atlantis platform in a fixed position throughout. The dwelling time was increased from 1 to 2.5s in increments of 0.5s per day. Each day 10 massed trials were given.

The transfer test took place 16 days after training, also in downstairs pool, and it consisted of the usual 60 second free swim with the platform absent.

9.3.1.2 Consolidation.

Run by Eva Roloff, Jacques Micheau and myself and rats implanted by Gernot Riedel. See Methods, ps. 27 and 32.

Rats were pre-trained and trained as above. One or 5 days after the end of training micropumps were implanted or acute infusions took place. Rats were tested 16 days later. See Figure 9.4.

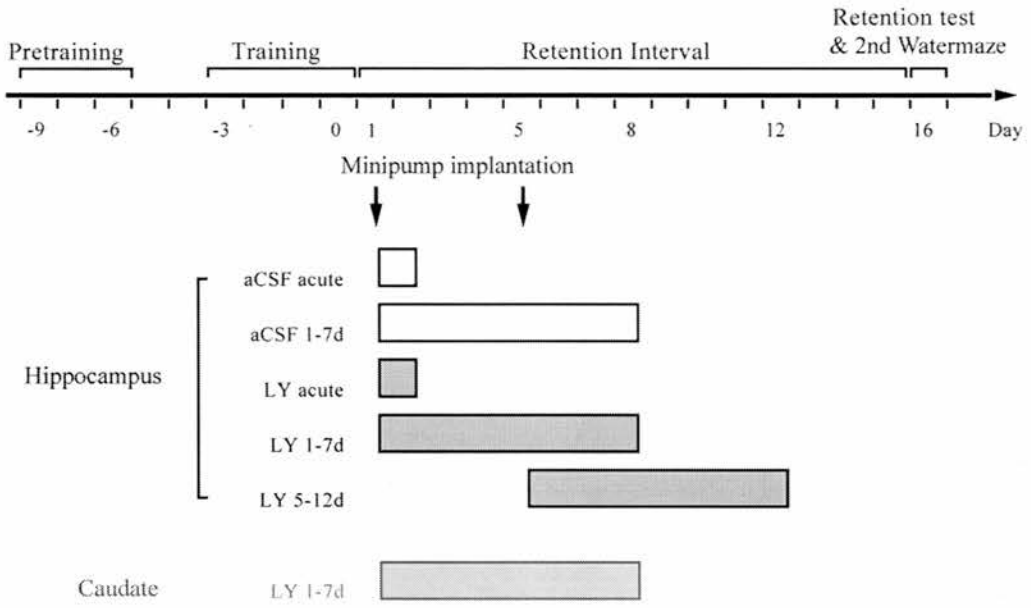

Figure 9.4: Schematic drawing of the experimental protocol used in the consolidation experiment. 2nd watermaze is included with the behavioural controls.

9.3.2 Results

9.3.2.1 Acquisition/ Retrieval

Acquisition and retrieval are presented here together because the design of the experimental protocol makes it necessary.

Thirteen rats were trained under aCSF and 11, under LY. During non-spatial pre-training performance is similar in both groups. This means that training can be approached by both aCSF and LY treated animals from identical grounds.

Analysis of training revealed that rats treated with LY during training were impaired in the acquisition of a spatial reference memory task in the watermaze in terms of escape latency (Fig. 9.5.a). The acquisition curve, in terms of escape latency for every 5 trials, was almost flat for LY treated rats while aCSF implanted animals showed a decrement in escape latency across days. Statistical analysis revealed an effect of Group ($F [1, 22] = 9.31$, $p < 0.01$). The slight increase in the aCSF group's

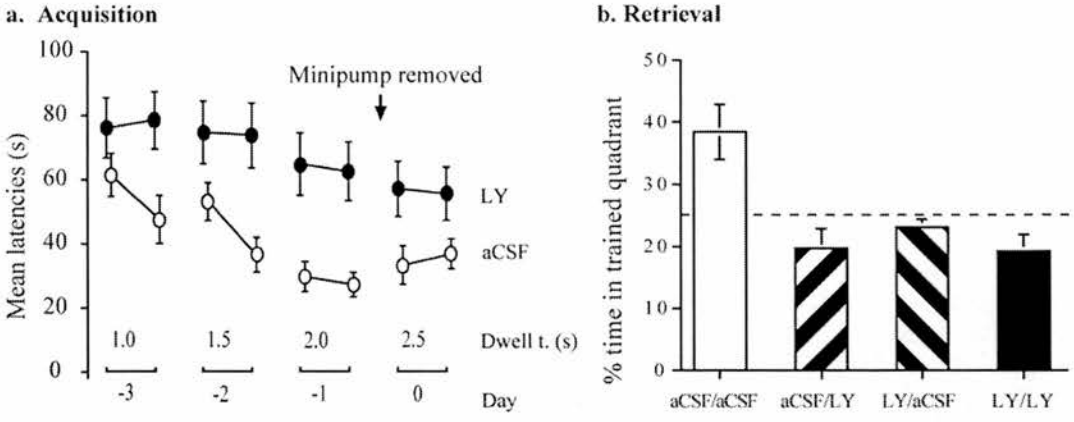

Figure 9.5: (a) Escape latency across days (each point represents 5 trials) and **(b)** performance during the transfer test as percentage time in training quadrant. (aCSF/LY): trained under aCSF/tested under LY.

latency on day 0 (4th day of training) was probably due to the increase in dwell time required for the platform to raise.

During the subsequent transfer test, as illustrated in Figure 9.5.b, only rats that were trained and tested under aCSF had a bias towards the training quadrant.

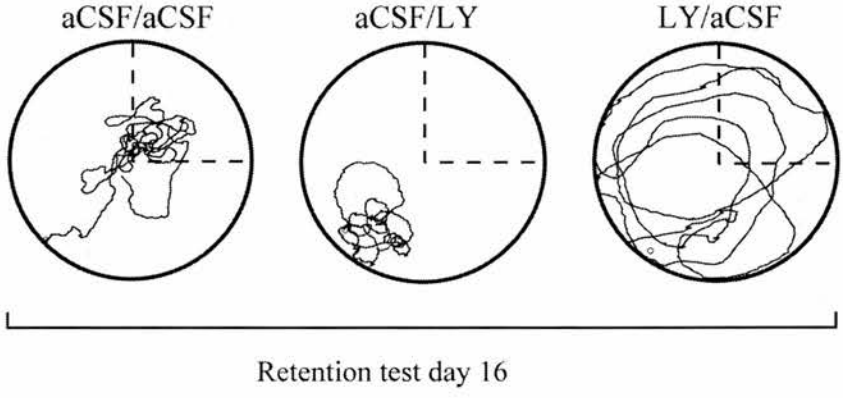

Figure 9.6: Representative swim paths taken during the transfer test. Conditions coded as before.

Rats trained under LY and tested under aCSF (impaired acquisition but normal retrieval capacity) were at chance. Rats trained under aCSF (normal acquisition) but tested under LY (impaired retrieval) displayed no preference for the training

quadrant. In fact, as these rats tended to start a dwell-like strategy as soon as they were put in the pool (Fig. 9.6), the highest percentage of time was spent in the opposite quadrant to that of training, where they were released. As expected rats that were trained under LY and tested under LY were impaired.

9.3.2.2 Consolidation

Nine and 8 rats constituted the acute and chronic aCSF groups respectively, 8 rats formed each of the chronic LY groups and 7 rats, the acute LY group.

Rats treated with aCSF, acutely or chronically, displayed a normal preference for the training quadrant: 35.8 ± 6.4 and 46.5 ± 6.7 % time respectively (Fig. 9.7.a). LY given acutely the day after training did not result in an impairment during the transfer test. This group spent 58.4 ± 7.4 % time in the training quadrant. However, LY given chronically for 7 days starting either 1 or 5 days after the end of training impaired performance in a transfer test as demonstrated by % time in training quadrant being close to or below chance: 15.8 ± 2.9 and 24.2 ± 6.9 respectively. Statistical analysis

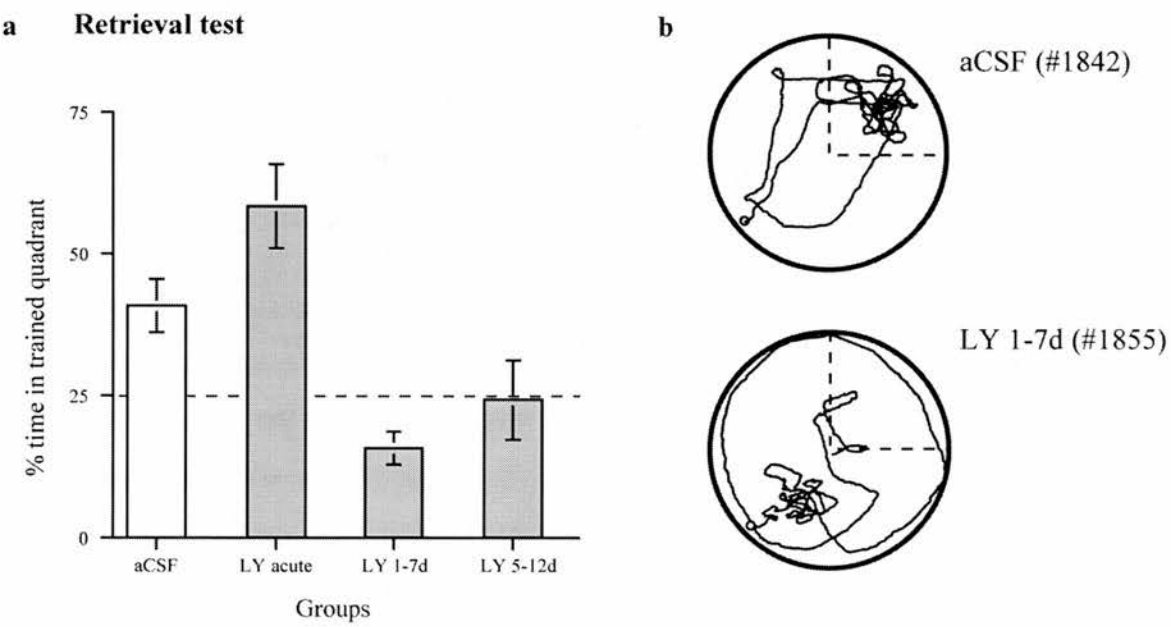

Figure 9.7: (a) Performance during transfer test in terms of percentage time in training quadrant. Acute and chronic aCSF groups are combined. **(b)** Representative swim paths taken during the transfer test.

revealed an overall Group effect ($F [4, 43] = 7.76$, $p < 0.0001$). Post-hoc Dunnett comparisons showed that both chronic LY groups were significantly impaired with respect to the combined aCSF group ($p < 0.05$). The acute LY group was, surprisingly, significantly better than the combined aCSF group ($p < 0.05$)

Rats chronically implanted with LY micropumps, although impaired in terms of preference for the training quadrant, were capable of displaying the dwelling strategy they acquired during training (Fig. 9.7.b).

9.3.3 Interim Discussion

Selective inactivation of the hippocampus during acquisition, consolidation or retrieval impaired performance during a retention test. These results suggest that hippocampus activity is necessary during each of the three processes.

Certain questions about the specificity of these results remain to be answered: are these results specific to the hippocampus-dependent aspects of the task? What happens if inactivation is induced in a different structure? Is hippocampal-dependent behaviour affected after the drug has been washed-out? And finally, is the impairment found during transfer test in the consolidation experiment a reflection of hippocampal dysfunction rather than of lack of consolidation?

A series of experimental controls is described in the following section, aiming to establish the task, structure, treatment-time and process-specificity of the behavioural results.

9.4 Behavioural controls

9.4.1 Methods

9.4.1.1 Task specificity.

Carried out by myself in its totality with the exception of the pilot studies that, in the form of a student project, were done with the help of 4 BSc students. See Methods, ps. 27, 32 and 34.

It was decided to use a non-spatial visual discrimination task in the watermaze to control for task specificity of the results outlined above. Two pilot studies took place in order to establish the design of a task that while being kept as similar as possible to the spatial task used in the behavioural studies needed to be hippocampus-independent. These pilot studies highlighted what has been indicated in the literature many times before: that rats find discriminations very difficult to acquire (Rescorla et al., 1985), especially when the 'error cost' is minimal.

The final protocol had the following shape:

Pretraining and training protocols were identical in terms of days and trials per day to those used in the behavioural experiments. This was also true of micropump implantation and testing (see Fig. 9.4). The groups used were equivalent to the aCSF 1-7d and LY 1-7d in Fig. 9.4

Training differed in that the 10 daily trials were spaced rather then massed and dwelling time was maintained at 0.5 seconds throughout training. Two distinct cues, one of which consistently signalled the platform position, hung above the water 20 cm from the wall in the centre of two adjacent quadrants. The platform was below the positive cue. Curtains were drawn around the pool and cue positions were varied between trials in order to prevent the rat from using a spatial strategy. A barrier joining the centre of the pool with the wall stood 45 degrees from each cue. This wall had the effect of forcing the animal to swim back to the centre of the pool if the incorrect cue was chosen and thus, increased the error cost.

9.4.1.2 Structure specificity.

Carried out by Jacques Micheau and Gernot Riedel. See Methods, ps. 27 and 32.

Protocol identical to that of Fig. 9.4. Only an LY 1-7d group was tested. Micropump implantations were made in the striatum rather than the hippocampus, the day after the end of training.

9.4.1.3 Treatment-time specificity.

Carried out by myself and rats implanted by Gernot Riedel. See Methods, ps. 27 and 32.

Rats were bilaterally implanted with 7 day aCSF or LY micropumps in the hippocampus. Two weeks later they were pre-trained, trained and tested in a reference memory task in watermaze (as in the general protocol, Fig 9.4).

9.4.1.4 Memory-process specificity.

Carried out by Jacques Micheau, Eva V. L. Roloff and rats implanted by Gernot Riedel. See Methods, ps. 27 and 32.

All rats run in the consolidation condition (LY and aCSF micropumps implanted 1 or 5 days after training and exhausted before transfer test) were trained in a new watermaze (upstairs) starting a few hours after the end of the transfer test performed downstairs (see Fig. 9.4).

The training consisted of 6 trials of a reference memory task using a standard hidden platform. The transfer test was done 5 hours after the end of training.

9.4.2 Results

9.4.2.1 Task specificity.

29 rats were trained in a visual discrimination task in the watermaze as described.

Rats performance during pre-training was no different from that obtained in the spatial experiments (data not shown). This is expected because the pre-training protocol is identical and performed with untreated animals. Rats quickly learned the significance of the hanging cue. This is followed by association between the cue and the platform position as revealed by appropriate dwelling over the platform position.

During training, when the two discriminanda (hanging cues) were introduced, none of the rats developed an obvious preference for the correct cue. Their choice of either side of the barrier in each trial was random as reflected by the fact that on the forth and last day of training the average of correct choices (out of 10 trials) across rats was 57 ± 3.5%.

The day after the end of training micropumps were implanted. From the original 29 rats, 3 were lost after surgery. This left 14 rats in the aCSF group and 12 rats in the LY group.

Sixteen days after surgery, rats were presented with a training trial look-alike with the exception that the platform was not present. As in a normal transfer test they were left to swim for 60 seconds.

The time spent in each of the cued quadrants was calculated as a % of time in both cued quadrants. Although both aCSF and LY treated rats show a tendency to spend more time around the rewarded cue, unfortunately this tendency is not different from chance for the LY treated rats (Fig. 9.8).

Rats implanted with aCSF minpumps spent, on average, 65 % ± 3 of their time in the correct quadrant and only 35 % ± 3 in the incorrect quadrant. The means for LY treated rats were 58 % ± 7 and 42% ± 7 respectively, which while not differing from

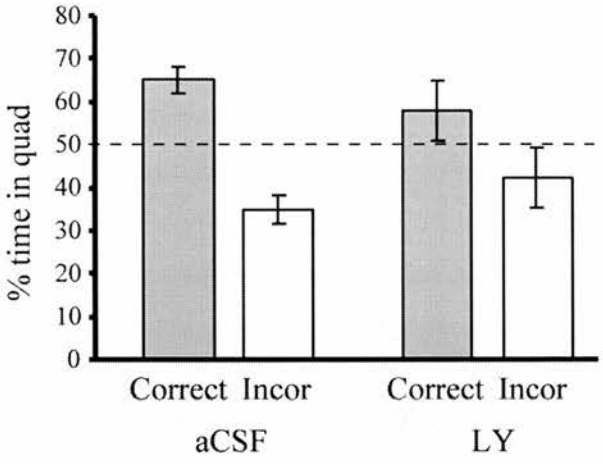

Figure 9.8: Time in each of the two cued quadrants during the transfer test as a proportion of the total time spent only in the two cued quadrants (thus chance is 50%). Correct: rewarded quadrant; Incor: non-rewarded quadrant. Horizontal line: chance level.

those of aCSF treated rats did not reflect a preference for the rewarded quadrant. Performance was more variable in the LY group (4 out of 12 rats spent more time in the incorrect quadrant while only 2 out of 14 did so in the aCSF group).

In an attempt to disclose whether the tendency towards the correct quadrant was higher at the beginning of the transfer test, % time in the rewarded quadrant after 15 and 30 seconds of transfer test was calculated. However, the results were similar to those of the full transfer test. Data not shown.

Correct first choice measurements revealed that the number of rats that chose to swim towards the correct cue was high in both aCSF (79 %) and LY (67 %) groups but, again, did not reach significant levels in the LY group (χ^2=1.33, p>0.2).

It has been suggested before that standard measurements of performance are not suitable to expose discrimination learning (Eninger, 1953; Mahut, 1954). This is very often latent and requires more subtle measurements to be exposed. It has been reported (Capaldi and Davidson, 1979) that, even when discrimination performance is still at chance, the latency to make an incorrect choice can be longer than the latency to make a correct choice. LY treated rats that made a correct choice, however, took as long to reach the correct cue (8 ± 2 seconds) as rats making an incorrect choice took to reach the incorrect cue (8 ± 1 seconds, see Fig. 9.9).

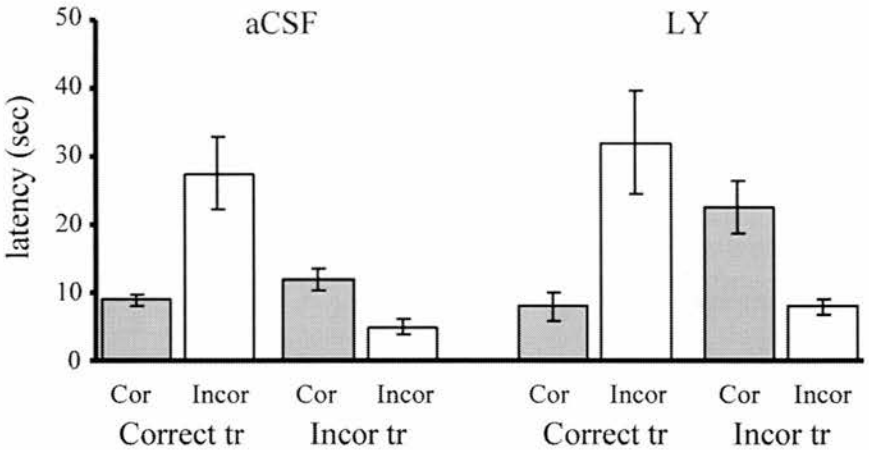

Figure 9.9: Latency to first reach the correct (rewarded) and incorrect cues during transfer tests depending on whether the correct cue (correct tr.) or the incorrect cue (incor. tr.) were chosen first.

Surprisingly the latency for aCSF rats is higher when choosing the correct cue in a correct choice (9 ± 0.8 seconds to reach the correct cue and 5 ± 1 to reach the incorrect cue). The latter could be explained either as a realization by the rats of a wrong choice (in an incorrect first choice) and completing the path at a higher speed or as a realization of a correct choice (in a correct first choice) and proceeding slowly towards it. However, another possibility is that a high proportion of the incorrect first choices are a result of a quick decision while a higher proportion of the correct first choices reflect a pause to look at both cues and decide on the correct one. The downside of this measurement is that the proportion of rats making a correct choice is different from that of rats making an incorrect choice in both groups.

9.4.2.2 Structure specificity.

The day after spatial training 9 rats were implanted with LY micropumps in the striatum. One rat died after surgery leaving a final 'n' of 8. An ANOVA revealed that during the transfer test, performed 16 days after surgery, this group performed as well as rats that had had aCSF micropumps implanted the day after training ($F[1, 14] < 1$).

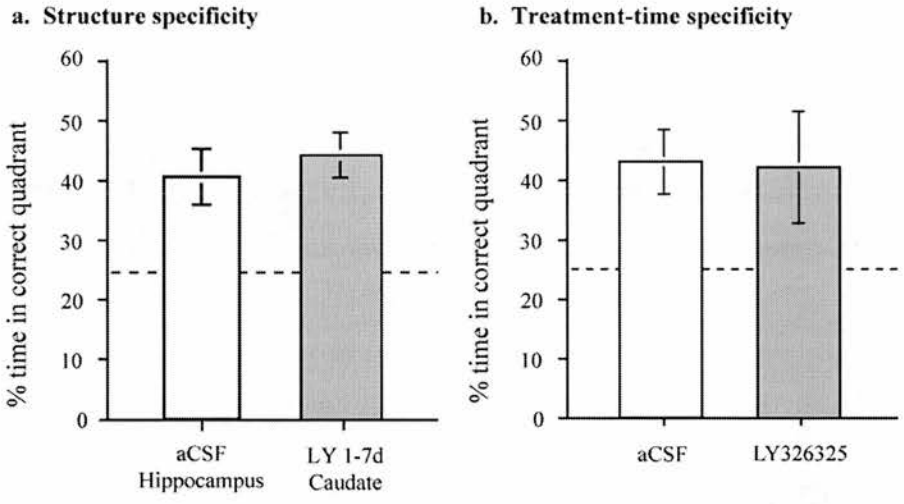

Figure 9.10: **(a)** Structure specificity and **(b)** treatment-time specificity as percentage time in training quadrant during transfer test. Dashed line represents chance.

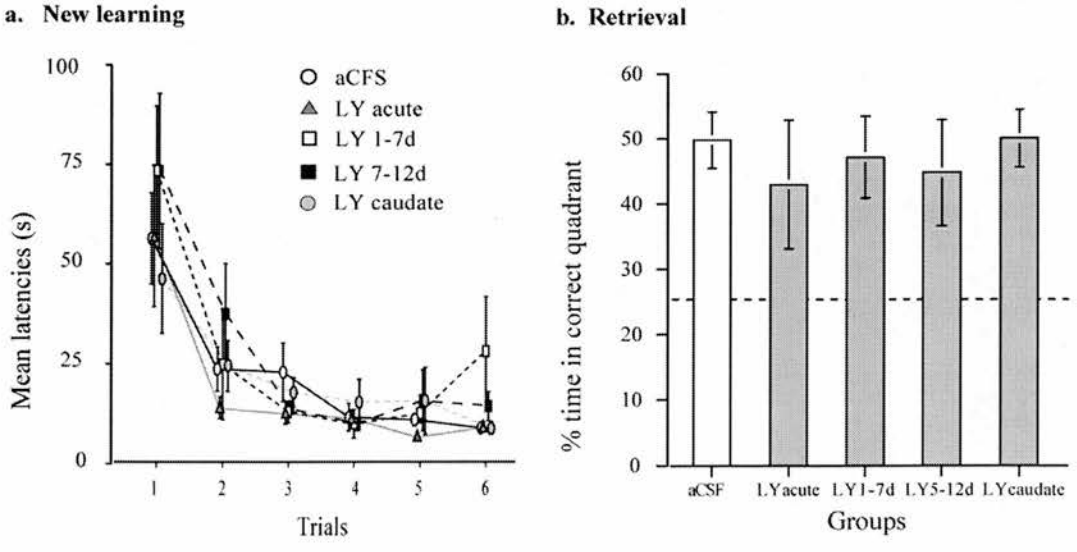

Figure 9.11: Memory-process specificity. **(a)** Acquisition and **(b)** transfer test performance in a new watermaze. Dashed line represents chance.

9.4.2.3 Treatment-time specificity.

In this control 16 rats were bilaterally implanted in the hippocampus with 7 day long micropumps (half aCSF, half LY). Micropumps were exhausted before any behavioural training began.

An ANOVA revealed no difference between groupsduring the final transfer test ($F[1, 14] < 1$) who performed above chance in the training quadrant (Fig. 9.10.b) with percentage time in training quadrant reaching $43.1 \pm 5.4\%$ and $42.2 \pm 9.4\%$ for aCSF and LY treated rats respectively.

9.4.2.4 Memory-process specificity.

The same groups tested in the consolidation experiment (LY infused between acquisition and retrieval) were given this further test.

All rats, aCSF and LY treated alike ($F = 1.8$), showed a decrease in escape latency across trials during training in a new watermaze (Fig. 9.11.a). In the transfer test performed 5 hours after the end of training all rats displayed a significant trend towards the correct quadrant (Fig. 9.11.b). No effect of group was found ($F = 1.97$, $p > 0.05$).

9.4.3 Interim Discussion

The series of behavioural controls successfully conclude that the results obtained in the behavioural experiments are an appropriate measure of hippocampal involvement in the processes of acquisition, consolidation and retrieval of a spatial memory task.

In the task-specificity control LY treated rats are not different from the aCSF group. Unfortunately their level of performance, although showing the right trend, is not strictly different from chance.

What is the meaning of the task-specificity results? This control was designed to establish whether findings obtained during the behavioural experiments reflected a hippocampus-dependent involvement in the different memory stages and not a

general effect of intra-hippocampal LY infusion on cognitive processing. In the non-spatial visual discrimination task, the lack of a clear bias for the correct cue in LY treated rats could reflect such a general cognitive deficit, but it could also be interpreted as an involvement of the hippocampus in this task. The task has not been used before and no information exists on whether lesions to the hippocampus would affect it or not. It was designed in the believe that it would not be hippocampus-dependent based on the fact that other visual discriminations are not (Marston et al., 1993; Deacon and Rawlins, 1996; but see Murray and Ridley, 1999). However, in retrospect, there are various factors that allow for the possibility that the task is not completely hippocampus-independent. For example, navigation is required during the learning of the task. The rat has to learn that only the hanging cues are relevant, that the platform is below one of them and that it can get from one cue to the other by returning to the centre of the pool and going around the barrier. We also know, from observing rats during the non-spatial pre-training, that rats trained in a watermaze have a very strong tendency to use spatial cues even when these are irrelevant. In addition the fact that none of the rats learn the discrimination during the four days of training is not ideal.

LY treated rats showed they remembered the importance of the two hanging cues and had a non-significant trend towards the rewarded one which they chose over the other one in 67% of the cases. Their performance was not different from that of aCSF treated rats but why was it not significantly above chance ? For the reasons stated above I believe this is more likely to be a result of hippocampus-dependent aspects of the task than a result of a general cognitive deficit caused by intra-hippocampal infusions of LY.

9.5 General Discussion

The study of hippocampal involvement in different memory stages is made possible by a novel compound, LY326325, which permits temporary inactivation of fast glutamatergic transmission in the hippocampus. Here, independent inactivation of either acquisition, consolidation or retrieval stages of a reference memory task in the watermaze revealed a role of the hippocampus in each of these memory stages.

9.5.1 Acquisition and retrieval

Hippocampal involvement in the process of acquisition is reflected in the fact that rats trained under LY do not show a decrease in escape latency.

Many lesion studies have presented evidence of a hippocampal involvement in acquisition of spatial memories before (e.g. Morris et al., 1982). Because the rats were not under the influence of LY during testing, for the first time, this impairment can be exposed as independent of retrieval.

Conversely hippocampal involvement in retrieval is evidenced in the impairment found in rats that, trained under aCSF and displaying a normal decrease in escape latency, were impaired when tested under LY (aCSF/LY group). However, as discussed in Chapter 8, it is possible that once a memory has been consolidated the hippocampus is no longer necessary for its retrieval. Results discussed below suggest that the process of consolidation has not terminated within the 16 days that follow training and precede retrieval. Had this process been completed, inactivation of the hippocampus during testing might have resulted in an unimpaired memory retrieval.

The LY/LY group could be comparable to a permanent lesion except for the important difference that, in this case, the hippocampus is active during the period of consolidation. Unsurprisingly, considering the above results, this group is impaired in both acquisition and retrieval. Whether the same result would have been obtained, had the type of memory not been dependent on the hippocampus for acquisition is not known. Although unlikely, it is possible that the hippocampus is essential for the consolidation of, otherwise, hippocampal independent memories.

9.5.2 The dwelling strategy

Comparison between aCSF/LY and LY/aCSF groups yields an interesting observation. Despite both being impaired during transfer test performance the pattern of their swimming paths is very different. While aCSF/LY rats display dwelling movements during the transfer test, LY/aCSF rats do not. This can be explained by the fact that while aCSF/LY rats had a chance to learn the dwelling strategy during

acquisition, LY/aCSF rats did not. It also implies that once the strategy has been learnt a functional hippocampus is not necessary to express it. Conversely, it has been argued that the fact LY/aCSF rats are impaired implies that the hippocampus is required for acquisition of the strategy. My view is that without a spatial capacity it is impossible for the rats to get a chance to reinforce any dwelling strategy that might have been learnt during the non-spatial pre-training. Dwelling at random anywhere in the pool would not bring the platform up, but, under LY influence, the rat would not be able to dwell any other way but at random. This unrewarded dwelling strategy would, thus, be extinguished.

Finally, the fact that rats can express the dwelling strategy even under the effect of LY (aCSF/LY animals) suggests that LY does not have a general effect on cognitive processes but rather a specific effect on hippocampal activity.

9.5.3 Consolidation

Hippocampal involvement during consolidation is illustrated by the fact that LY infusion for longer than 1 day generates an impairment as measured during a drug-free retention test. The beauty of this experiment resides in the fact that the hippocampus is functional during both acquisition and retrieval, and therefore all the time that the rat's behaviour is being monitored.

An important conclusion to be drawn from these results is that the deficit observed in amnesias is not necessarily one of retrieval. It was discussed in the previous chapter that, ultimately, in order for the memory to become independent of the hippocampus, retrieval had to be possible without this structure. Thus, amnesias provide no evidence that a consolidation process (understood here as independent of retrieval) is actually happening within the hippocampus. Here it is demonstrated that, even when the capacity of retrieval is intact, retrieval of memories is impaired. Therefore, a process of consolidation, independent of retrieval, does take place. Moreover this process is autonomous, in the sense that no externally cued reactivation of the memory took place during the interval between acquisition and testing.

This experiment also demonstrates that, consolidation takes longer than 16 days. Therefore, as pointed out in the previous section, in order to establish whether, once the process of consolidation has terminated, memories can be retrieved with an inactivated hippocampus two more experiments would be required. First, one would need to establish the end of the process of consolidation for this particular reference memory task. Second, in a parallel experiment, one would need to inactivate the hippocampus once the memory has been consolidated and test the rat's capacity for retrieval.

Although the impairment observed in rats whose hippocampus was inactivated during the so called consolidation period has been interpreted as a deficit in memory consolidation, it is also possible that the LY infusion affects a site of storage and causes the memories to be erased. Storage is intuitively understood as a process that does not require activity to be maintained. The truth, however, is that the mechanism by which memories are stored in the brain is not known. Memory storage could well require an active process that depends on normal activity in the hippocampus. Even in the case of a more traditional storage view, this could depend on the synthesis of a particular molecule, whose turnover could be affected by neuronal inactivation by LY.

This difficulty to distinguish between a storage process and a consolidation process suggests that memory consolidation needs to be understood in a broader way as the process by which memories become independent of the hippocampus, regardless of the nature of this process. In this case it is irrelevant whether LY has affected an active strengthening of the memories or the molecular code that represented their storage. The fact is that inactivating the hippocampus impairs the retrieval of those memories which are, therefore, still hippocampus-dependent. In this case memory consolidation can involve a hippocampus-dependent process by which memories become stable in a different structure such as the neocortex but also a process by which memories are stabilized within the hippocampus. Once the process is completed a lesion to the hippocampus should have no effect on the memories whether they are stored in neocortex only or also in the hippocampus.

9.5.4 Acute versus chronic LY infusion

Acute infusion of LY the day after the end of training not only has no deleterious effect on performance but, indeed, it has a facilitatory effect as illustrated by the fact that animals treated acutely with LY perform better than aCSF rats.

The lack of impairment following acute LY infusion suggests that consolidation needs more than one day to occur, as supported by the findings that blocking the hippocampus for 7 days starting either 1 or 5 days after training does successfully impair performance in the transfer test that follows. It also means that interrupting the process momentarily does not prevent it from continuing later on. Would this still apply had the acute infusion been given in the middle of the 16 days interval? If not, it could be argued that the consolidation process dependent on fast glutamatergic transmission does not begin immediately after the end of acquisition; maybe because at that point there is no indication that acquisition has actually finished.

Another possible explanation of the different result obtained from acute and chronic inactivation is that the relevant factor is the time between inactivation and testing. This is 15 days for the acutely infused rats versus 9 or 4 days for the chronically infused animals. Assuming that consolidation can be restarted even when the hippocampus has been inactivated for long periods of time, it is possible that 15 days, but not 9 or 4, is enough for the process of consolidation to take place. This hypothesis would be in agreement with human cases of temporary amnesia in which memories acquired soon before the trauma are recovered after the recession of amnesia (Barbizet et al., 1970).

9.5.5 Conclusions and further questions

The involvement of the hippocampus in the process of acquisition, consolidation (in its broadest meaning) and retrieval has been established.

These results confirm hippocampal involvement in a consolidation process that is independent of retrieval. Also, that this consolidation process either starts between 24 (first LY infusion) and 48 hours after the end of acquisition period or is not

interrupted by inactivation lasting less than 24 hours. Finally, that the process, be it strengthening of traces or storage, can be autonomous, i.e.: does not require external re-activation of the memory.

Certain questions remain unanswered. For example, it is not known whether the involvement of the hippocampus in retrieval is dependent on the fact that the process of memory consolidation has not finished. Also, the experiments here described did not determined the length of the process of consolidation. These two questions are related and constitute the subject a follow-up study, described in Chapter 10.

Chapter 10

Time-independent role of the hippocampus in spatial memory explored through a novel reminding protocol

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Chapter 10

Time-independent role of the hippocampus in spatial memory explored through a novel reminding protocol

10.1 Introduction

While consolidation experiments in animals using tasks other than the watermaze have found evidence for a time-dependent role of the hippocampus in memory (Winocur, 1990; Kim and Fanselow, 1992; Anagnostaras et al., 1999), lesions to the hippocampus at different time-intervals after acquisition of a reference memory task in the watermaze have consistently resulted in a flat gradient of retrograde amnesia (Bolhuis et al., 1994; Mumby et al., 1999a). It is possible that the spatial memories of the kind acquired during a reference memory task in the watermaze are permanently dependent on the hippocampus. However, it is also possible that such a complex memory, involving not only information on a spatial location but also on how to navigate to it, cannot be expressed, in the absence of the hippocampus, during a single traditional transfer test performed weeks after acquisition.

To test this possibility, in a pilot study involving rats with both sham and hippocampal lesions, it was agreed that rats would receive a series of transfer tests, spaced by one hour and each rewarded at the end of it by the raising of the Atlantis platform in the original quadrant. The results were spectacular. During the first transfer test of the day, rats displayed no spatial bias. The Atlantis platform was raised at the end of the 60 seconds and the rats were allowed to find it and rest on it for 30 seconds. Rats were tested again one hour later. During that second transfer

test, all the groups, including the lesioned one, spent an unusually long time in the training quadrant. The lesions involved the septal 60% of the hippocampus.

In this chapter I present a study aiming to further explore, using this novel reminding protocol, whether the memory for the platform location is lost after complete hippocampal damage or merely inaccessible. An orthogonal question is also posed. Damage to the hippocampus in human cases of amnesia is often partial, a fact that is generally ignored when discussing the different memory phenotypes of these patients. In this study the retrograde memory capacity of rats with complete hippocampal lesions is compared with that of rats with only septal hippocampal lesions.

A between subject design is used to test the effect of hippocampal lesions on recent and remote spatial memories and a modified testing protocol is applied in order to enhance access to a memory that might be temporarily inaccessible. Additional groups are used to control for the possibility that the reminder protocol might result in new learning rather than reactivation of an old memory.

This study has been performed together with Dr Steve Martin. He has taken care of the watermaze side of the study while I have focused on the surgery, perfusions and posterior assessment of the lesion. As in previous studies, Jane Knox sectioned, mounted and stained the brains.

10.2 Methods

10.2.1 Lesions

Hippocampal ibotenic acid lesions (see Methods, p.27) were given either during the two days immediately after training (recent condition) or 6 weeks later (remote condition). Testing took place two weeks after the lesions.

The lesions were either complete hippocampal lesions or aiming to the septal 60% of the hippocampus (thus sparing the temporal 40%). The size of the partial lesions was

intended to replicate the extent of the LY326325- dependent inactivation. Sham lesions were also performed.

10.2.2 Behavioural training and testing

The protocol was as similar as it was possible to the one used in the experiment presented in Chapter 9, in order to facilitate comparisons. Some modifications, however, were deemed necessary. The protocol is as described in Figures 10.1 and 2.

- Two training to lesion time-intervals were used: 1 day (recent condition) and 6 weeks (remote condition).

- Non-spatial pre-training was identical to that given to rats in the experiments described in Chapter 9, but it was reduced to one day.

Training (Fig. 10.1):

- 10 trials per day in 2 session of 5 trials each, for 4 days.

- The dwelling time required to raise the platform was 0.5 second.

Testing- Day 1 (Fig. 10.2):

- Memory was explored with a traditional 60 second long transfer test, using the Atlantis platform kept at the bottom of the pool.

- At the end of this time the Atlantis platform was raised in the original training position and the rat was allowed to climb to it and stand there for 30 seconds. Two more such transfer tests were given spaced an hour apart. This procedure is called the 'original testing protocol' and was given to half the animals.

- The other half of the animals were given the 'novel testing protocol'. At the end of transfer test 1 the platform was raised in the quadrant opposite to that rewarded during training. In transfer test 2, memory for the original training

position after one reminder can be compared with memory for a novel platform location after one exposure to this position.

Testing- Day 2: Data not reported in this chapter

- Rats that received the original testing protocol were given the novel protocol and *vice versa*.

Testing- Day 3 (Fig. 10.2):

- To control for the context dependency of the results. Three rewarded transfer tests were given to all rats in a different pool in a new environment. This is referred to as 'new learning'.

Throughout testing rats were released in the adjacent quadrants (left and right adjacent quadrants were counterbalanced), rather than in the opposite quadrant as it is traditional. The position of the platform in the quadrant opposite to training in the novel testing protocol made this necessary.

The rationale for these features of the design was, in turn: a) the use of a sufficiently long training to lesion interval to see the effect of the putative consolidation process; b) spaced trials to facilitate memory over such long interval; c) short dwelling time to prevent the expression of the dwelling strategy during the transfer test from overriding the spatial bias; d) the use of a novel test and new learning to control for the possibility that the reminder effect is really a very rapid new learning and test whether this is context dependent or not.

A small note in nomenclature is necessary here. Group refers to the type of hippocampal lesion, which can be sham, partial or complete. Time-interval refers to the time between training and lesion and, thus, to the recent and remote conditions. Original/Novel refers to the type of testing protocol: original and novel.

Fig.10.1

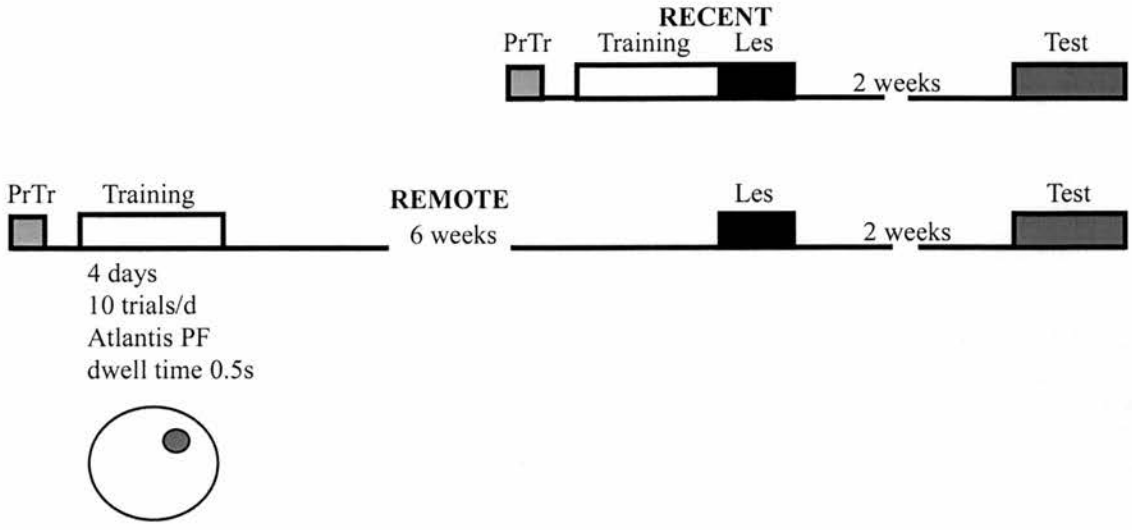


Fig.10.2

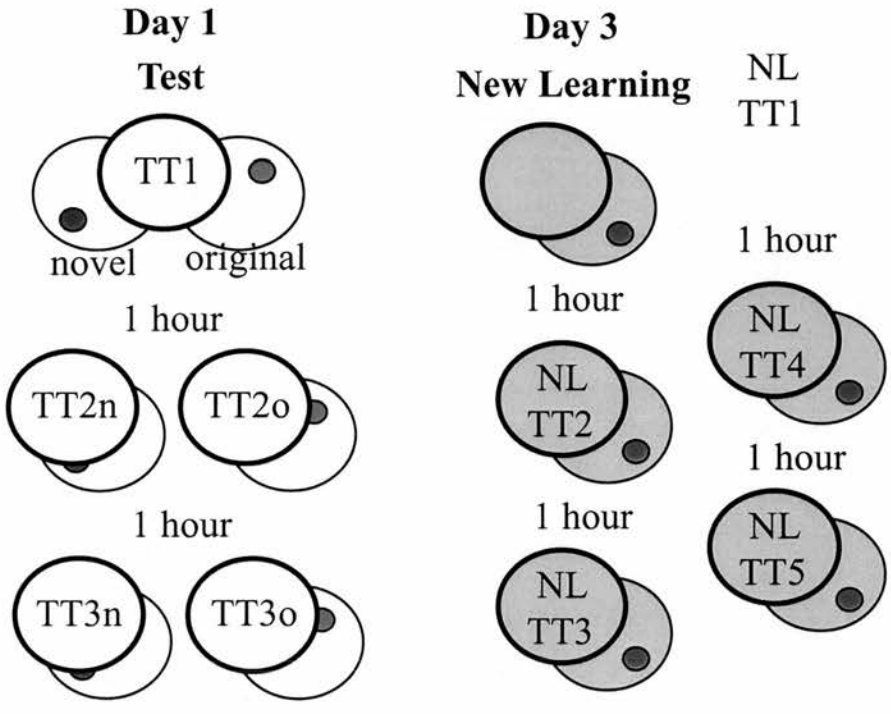


Figure 10.1 and 2: General protocol (1) and detailed testing procedure (2). In the original condition (o) rats are rewarded, at the end of each transfer test (TT1 to 3) in the training position (red). In the novel condition (n) rats are rewarded in the opposite quadrant (blue). New learning (NL) took place in a novel environment (grey). PrTr: training; Les: lesion is given.

10.3 Results

10.3.1 Histology

Of the 126 rats that were trained, 118 passed the set criterion and received sham, partial or complete hippocampal lesions. From these 3 died after surgery and 8 were discarded after histological analysis. The remaining 107 were divided as follows.

In the recent condition 10 rats belonged to the sham original and 10 to the sham novel groups, 10 and 8 belonged to the partial original and novel groups respectively and 9 and 10 to the complete original and novel respectively. The spared volume of tissue of the partial lesioned rats ranged between 22 and 50% and averaged 31 ± 2%. There was no difference between original and novel spared tissue.

In the remote condition 9 rats belonged to the sham original and 9 to the sham novel groups, 6 and 8 belonged to the partial original and novel groups respectively and 8 and 10 to the complete original and novel respectively. The spared volume of tissue of the partial lesioned rats ranged between 28 and 50% and averaged 41 ± 2%. There was no difference between original and novel spared tissue.

10.3.2 Acquisition

Rats were assigned to groups after acquisition was completed such that the average escape latency on the last day of training was equal between groups. Accordingly no effect of Time-interval ($F < 1$), Group ($F < 1$) or Original/Novel ($F [1, 94] = 1.08$, $p > 0.05$) was found in an overall analysis of escape latencies per trial. Latencies decreased during training (Fig. 10.3) and an effect of Trial was obtained ($F [39, 3666] = 42.63$, $p < 0.001$).

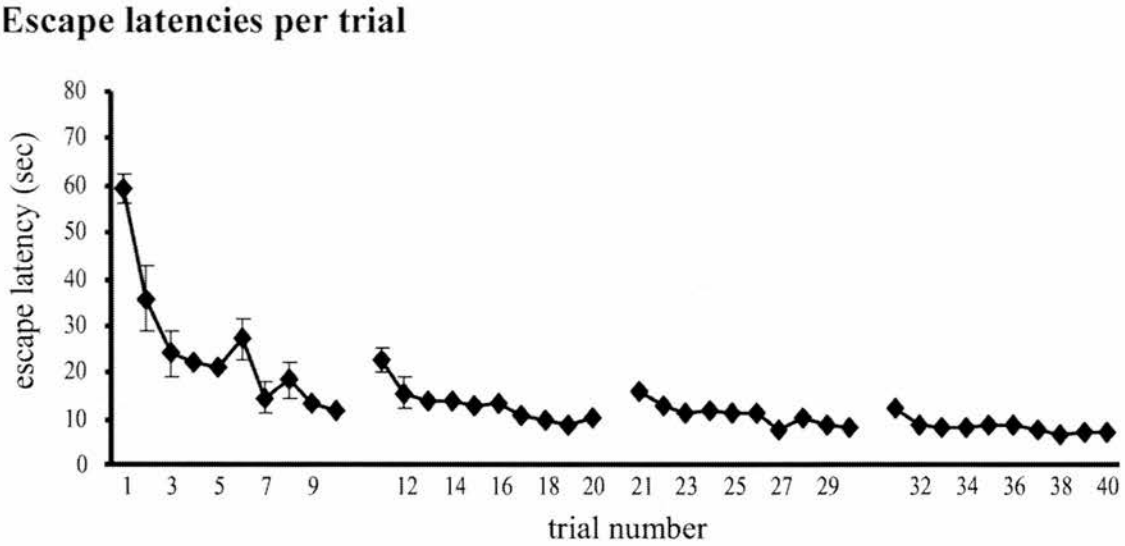


Figure 10.3: Escape latencies during acquisition across trials. Average of all rats.

10.3.3 Memory

The degree of spared memory is measured here by performance on transfer test 1 before any reminder is given. Subjects assigned to the original and novel conditions are, therefore, still together in one group.

The recent condition is analyzed first. Figure 10.4 represents performance during the duration (60 seconds) of the first transfer test in terms of percentage time in training and opposite quadrants. The reason to plot only these two quadrants, ignoring the adjacent ones, is that time spent in the latter was artificially high. Rats that have not visited the pool for several days have a tendency to swim were they are released which, in this case and as required by the protocol, happened to be the adjacent quadrants. For this reason the target and opposite quadrants are chosen for analysis. An overall analysis of the first transfer test revealed an effect of Group ($F [2, 54] = 15.73$, $p < 0.001$), an effect of Quadrant ($F [1, 54] = 23.25$, $p < 0.001$) and a Group by Quadrant interaction ($F [2, 54] = 10.25$, $p< 0.001$). This effect of Quadrant, reflecting a spatial bias towards the training quadrant, was also found after analysis

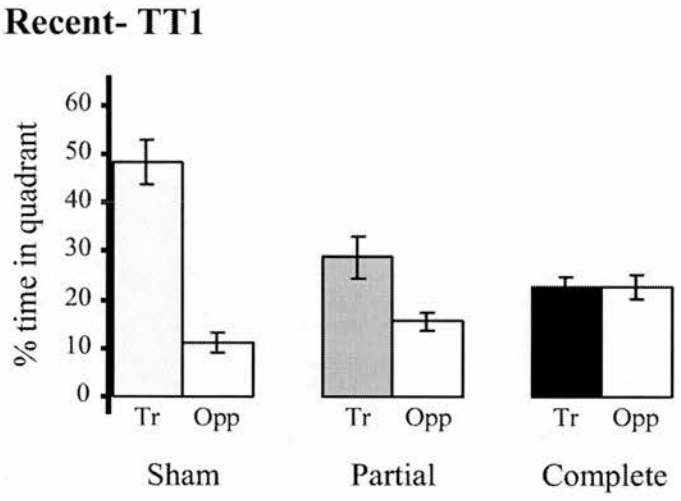


Figure 10.4: Recent condition, % time in training (coloured) and opposite (white) quadrants for the sham, partial and complete lesion groups.

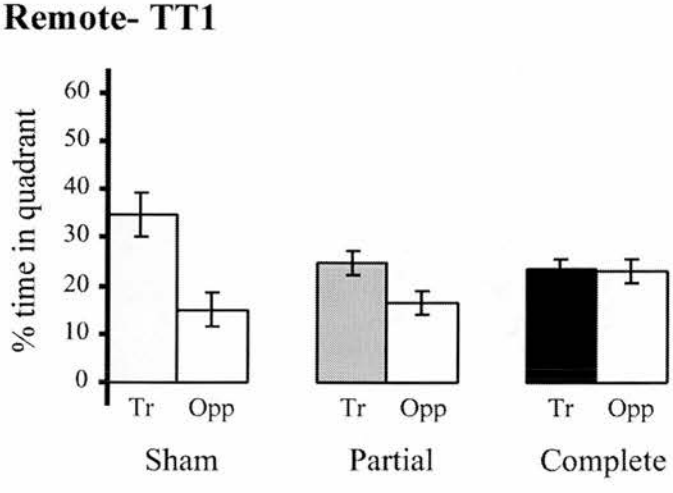


Figure 10.5: Remote condition, % time in training (coloured) and opposite (white) quadrants for the sham, partial and complete lesion groups.

of the sham group ($F [1, 39] = 50.26$, $p < 0.001$) and partial group alone ($F [1, 35] = 7.35$, $p = 0.01$), but not after analysis of the complete group alone ($F < 1$).

An analysis of performance in transfer test 1 in the remote time-interval revealed a similar pattern of results (Fig. 10.5). There was an effect of Group ($F [2, 47] = 3.27$, $p < 0.05$), an effect of Quadrant ($F [1, 47] = 7.29$, $p = 0.01$) but no Group by Quadrant interaction ($F [2, 47] = 2.89$, $p = 0.065$). An effect of Quadrant was found after analysis of the sham group alone ($F [1, 35] = 11.1$, $p < 0.001$) and the partial group alone ($F [1, 27] = 5.33$, $p < 0.05$) but not after analysis of the complete group alone ($F < 1$). The lack of Group by Quadrant interaction is probably due to the similarity in % time in training quadrant between the partial and the complete lesion groups, despite the fact that the former, but not the latter, displayed a spatial bias.

No difference was observed between the recent and the remote time-intervals when analyzed in terms of % time in training quadrant only ($F [1, 106] = 2.6$, $p > 0.05$; Fig. 10.6). This graph reveals some forgetting in the sham lesion group.

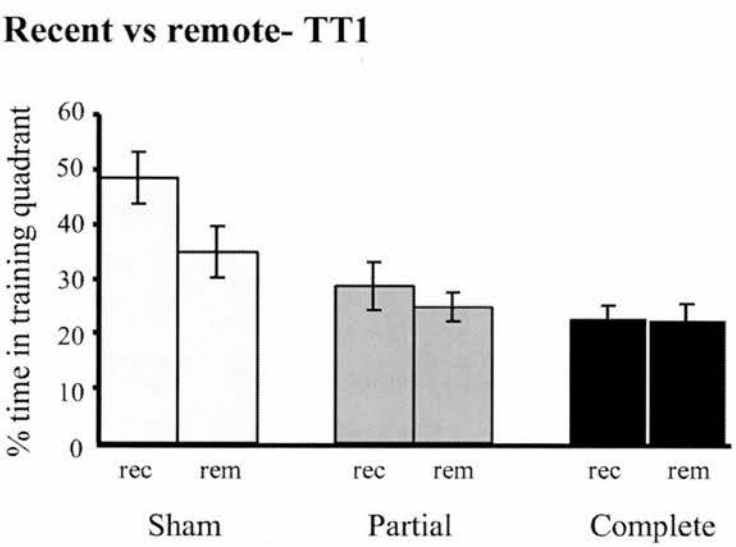


Figure 10.6: Recent versus Remote condition, % time in training quadrant in the recent (left bar) and remote (right bar) conditions for the sham, partial and complete lesion groups.

Consistent with previous studies (Bolhuis et al., 1994; Mumby et al., 1999), rats with no hippocampus were impaired at both time-intervals. This flat gradient of retrograde amnesia suggests the lack of a time-dependent role of the hippocampus in spatial memory. Rats with only temporal hippocampus spared, on the other hand, displayed a spatial bias, albeit limited, reflecting some sparing of memory. Sham animals remembered at all time-intervals but showed forgetting with time.

Does the lack of spatial bias in complete lesioned rats reflect a complete loss of memory? According to the standard theories of memory consolidation, the memory trace stored in the neocortex should suffice, in the remote condition, provided the process of consolidation has ended. It is possible however that this memory, the hippocampal connection being lost, needs to be reactivated.

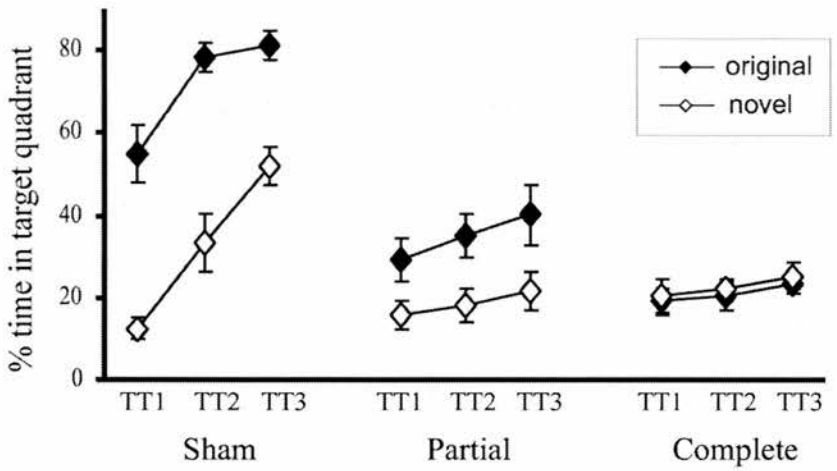
10.3.4 Reminding

As discussed in the methods, a difference between the original and the novel conditions is interpreted as expression of a previously existing memory. If rats with complete lesions were to develop a spatial bias after one reminder of the original location but not after an exposure to the novel location, one could say that the memory of the original location was spared but needed to be reactivated in order to be expressed; and that this reactivation was unlikely to be due to one-trial learning.

What happens in the recent condition? Figure 10.7.a illustrates the % time in the target quadrant at each transfer test for the original and the novel conditions separately. This applies also to the first transfer test where, unlike in the previous section, rats that were rewarded with the platform in the original quadrant and those rewarded in the novel quadrant are grouped apart.

The data plotted in Figure 10.7.a confirmed memory (transfer test 1) in the sham and partial lesion groups and improvement with reminders, although this was only a tendency in the partial lesion group. Rats with complete hippocampal lesions were impaired at all transfer tests. The sham group also displayed learning of the novel platform position. Accordingly, an overall analysis revealed an effect of Group ($F [2, 51] = 39.65$, $p < 0.001$), an effect of Original/Novel ($F [1, 51] = 34.71$, $p < 0.001$)

Recent- original vs novel across TTs



10.7.a: Recent condition, % time in target quadrant (original and novel quadrant for original and novel conditions) across transfer tests. For each group each data point represents, form left to right, the first, second, and third transfer test.

Recent- original vs novel across TTs (target-opp)

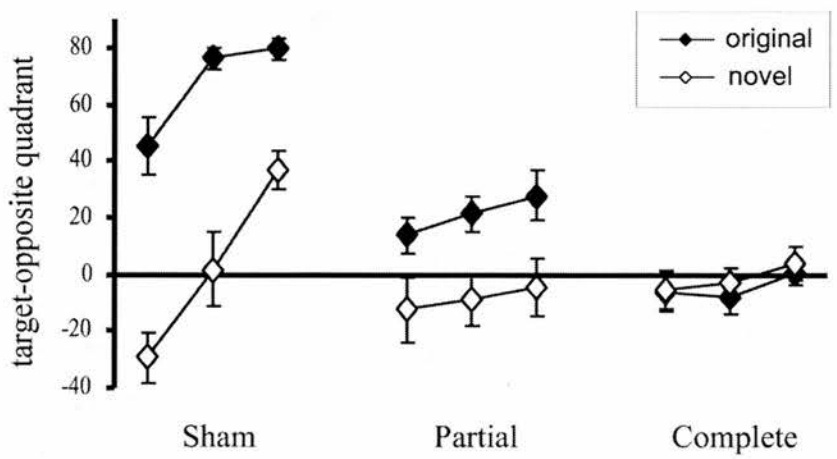


Figure 10.7.b: Recent condition, % time in target minus % time in opposite quadrant for the original and novel conditions across transfer test 1 to 3 for each lesion group.

and a double interaction ($F [2, 51] = 15.44$, $p < 0.001$). Here the Original/Novel effect refers to differences between the original and the novel conditions. There was also an effect of Transfer Test ($F [2, 102] = 31.1$, $p < 0.001$) and a Group by Transfer Test interaction ($F [4, 102] = 11.2$, $p < 0.001$) but no Original/Novel by Transfer Test interaction ($F < 1$).

Analysis of shams alone revealed an effect of Original/Novel ($F [1, 18] = 53.06$, $p < 0.001$) and an effect of Transfer Test ($F [2, 36] = 34.2$, $p < 0.001$). Partial lesion animals, on the other hand, showed an effect of Original/Novel ($F [1, 16] = 6.28$, $p < 0.05$) but no effect of Transfer Test ($F [2, 32] = 3.01$, $p > 0.05$). Analysis of the complete lesion group revealed no effect of either Original/Novel ($F < 1$) or Transfer Test ($F [2, 34] = 2.08$, $p > 0.05$).

Figure 10.7.b represents the difference between target and opposite quadrant at all transfer tests. A spatial bias results in positive performance in this data plot. The graph reveals a similar pattern of results to those of Fig 10.6 and some additional information. For example, sham animals rewarded in the novel quadrant acquired a positive bias towards this quadrant only on transfer test 3. The increase in % time in target quadrant observed from transfer test 1 to 2 was the result of a group tendency to decrease the search over the original training quadrant. Also, the data revealed that the partial lesion group rewarded to the novel quadrant did not acquire a spatial bias for this quadrant. Rats with complete hippocampal lesions showed no improvement over time in either the original or novel conditions.

Analysis of the remote time-interval, in terms of % time in target quadrant (Figure 10.8.a), revealed a similar overall pattern of results to that obtained in the recent condition. Memory was confirmed in the sham and partial groups and the lack of it in the complete group. Improvement with reminders was observed in the sham and, to a lesser extent, in the partial

Remote- original vs novel across TTs

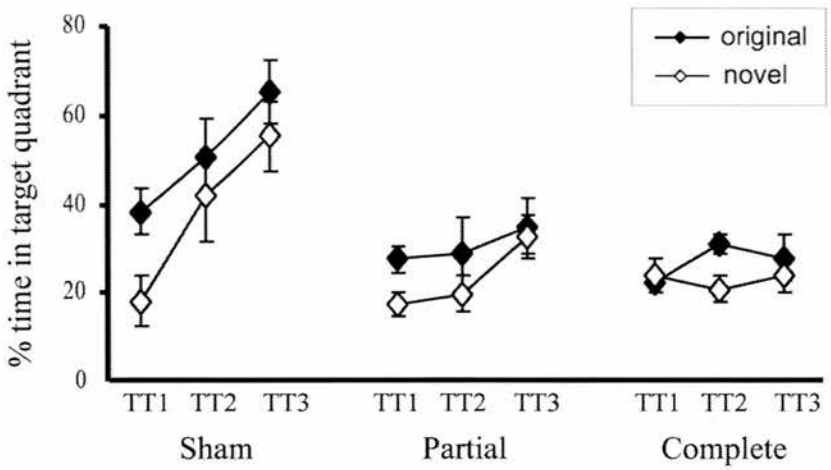


Figure 10.8.a: Remote condition, % time in target quadrant (original and novel quadrant for original and novel conditions) across transfer tests. For each group each data point represents, form left to right, the first, second, and third transfer test.

Remote- original vs novel (target-opp)

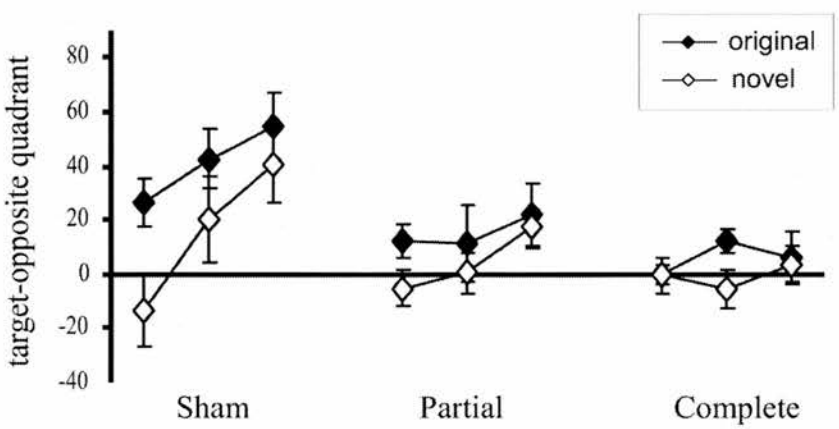


Figure 10.8.b: Remote condition, % time in target minus % time in opposite quadrant for the original and novel conditions across transfer test 1 to 3 for each lesion group.

group. Surprisingly and unlike in the recent condition, animals with partial lesions rewarded to the novel quadrant revealed an improvement over time. There was an effect of Group ($F [2, 44] = 10.97$, $p < 0.001$) and an effect of Original/Novel ($F [1, 44] = 4.18$, $p < 0.05$). However, no Group by Original/Novel interaction was found ($F < 1$).

Analysis of individual groups revealed no effect of Original/Novel (shams: $F [1, 16] = 1.99$, $p > 0.05$; partial: $F [1, 12] = 1.57$, $p > 0.05$; and complete: $F [1, 16] = 1.24$, $p > 0.05$). This lack of effect, however, does not shed doubt on the memory for the original quadrant observed during the first transfer test and analyzed in the previous section. An effect of Transfer Test was found in sham and partial lesion groups ($F [2, 32] = 18.86$, $p < 0.001$; and $F [2, 24] = 5.95$, $p < 0.01$; respectively). No effect of transfer test was obtained in the complete lesion group ($F < 1$).

Figure 10.8.b illustrates the difference between target and opposite quadrant during the different transfer tests. The pattern of results is similar to that on the previous figure (Fig. 10.8.a) and reveals a capacity in the groups with hippocampal sparing for learning of a novel platform. A spatial bias towards the novel quadrant is observed in shams by transfer test 2 and in the partial lesion group by transfer test 3.

The good performance of animals with complete lesions during transfer test 2 in the original condition could be interpreted as reactivation of a non-hippocampal dependent memory trace. This result is, however, difficult to understand because it does not hold in the consecutive transfer test 3 and because no effect of Transfer Test was found in this group.

The possible differences between the recent and remote conditions are further explored.

Figure 10.9 represents percentage time in training quadrant across the different transfer tests for the original condition only. An overall analysis of these data revealed no effect of Time-interval ($F [1, 46] = 2.64$, $p > 0.05$), an effect of Group ($F [2, 46] = 38.21$, $p < 0.001$) and an effect of Transfer Test ($F [2, 92] = 22.91$, $p < 0.001$). Interactions were found between Time-interval and Group ($F [2, 46] = 4.39$,

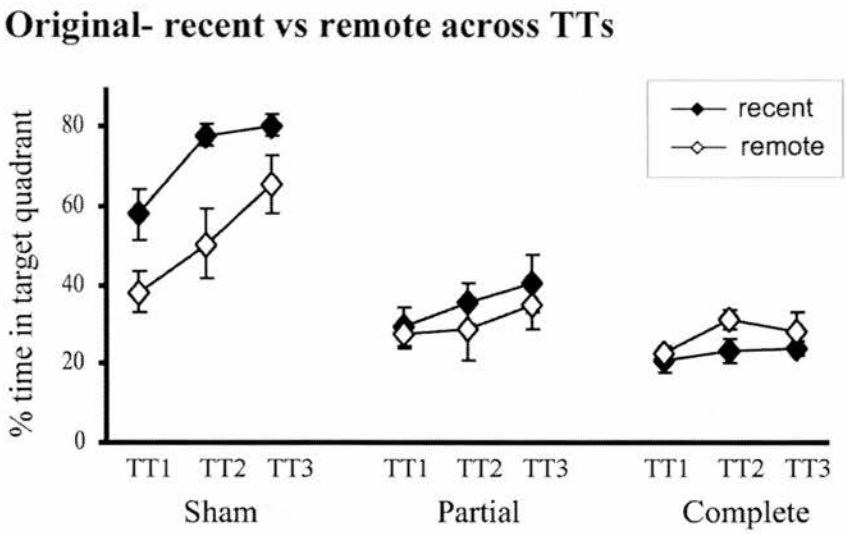


Figure 10.9: Recent versus remote, % time in target quadrant for the original condition across transfer test 1 to 3.

$p < 0.05$) and Group and Transfer Test ($F [4, 92] = 6.01$, $p < 0.001$). An analysis of shams only revealed an effect of Time-interval ($F [1, 17] = 7.55$, $p < 0.05$), an effect of Transfer Test ($F [2, 34] = 25.23$, $p < 0.001$) but no Time-interval by Transfer Test interaction ($F [2, 34] = 1.55$, $p > 0.05$). An analysis of partially lesioned animals only revealed no effect of Time-interval ($F < 1$), no effect of Transfer Test ($F [2, 28] = 2.87$, $p > 0.05$) and no interaction ($F < 1$). Analysis of the complete lesion group revealed no effect of Time-interval ($F [1, 15] = 3.49$, $p > 0.05$), no effect of Transfer Test ($F [2, 30] = 2.41$, $p > 0.05$) and no interaction ($F [2, 30] = 1.1$, $p > 0.05$).

Thus, the reminder protocol does not bring out a time-dependent role of the hippocampus in memory for the task at hand. The only difference between recent and remote memory is observed in shams, who forget over time.

How are rats rewarded to a novel location affected by the time-interval? Figure 10.10 illustrates the percentage time spent in the novel target quadrant across transfer tests for the different groups in the recent and remote conditions. An overall analysis revealed no effect of Time-interval ($F [1, 49] = 1.31$, $p > 0.05$), an effect of Group ($F [2, 49] = 8.48$, $p = 0.001$) and an effect of Quadrant ($F [2, 98] = 28.56$, $p < 0.001$).

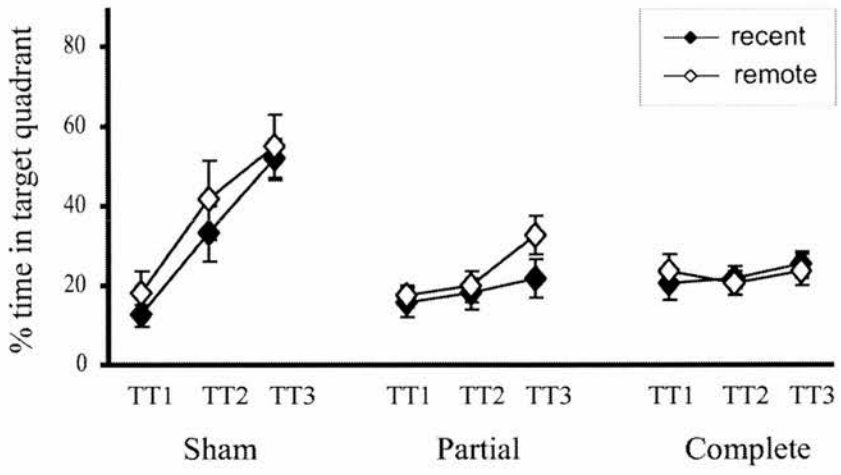


Figure 10.10: Recent versus remote, % time in target quadrant for the novel condition across transfer test 1 to 3.

There was a Group by Transfer Test interaction ($F [2, 98] = 12.4$, $p < 0.001$). Analysis of individual groups exposed no effect of Time-interval ($F < 1$ for the sham and complete lesion groups and $F [1, 14] = 1.21$, $p > 0.05$ for partial lesion group). An effect of Transfer Test was found in the sham and the partial lesion groups ($F [2, 34] = 26.15$, $p < 0.001$ and $F [2, 28] = 6.02$, $p < 0.01$, respectively).

Thus, shams and rats with partial hippocampal sparing showed an increase in time in the novel quadrant after being rewarded in it. This increase in the partial lesion group, although more apparent in the remote condition is, however, not sufficient to bring out a difference between recent and remote time-intervals

10.3.5 New learning in a novel context

Two days after testing all animals received three rewarded transfer tests in a novel pool in a different room to test for context-specificity of the previous results.

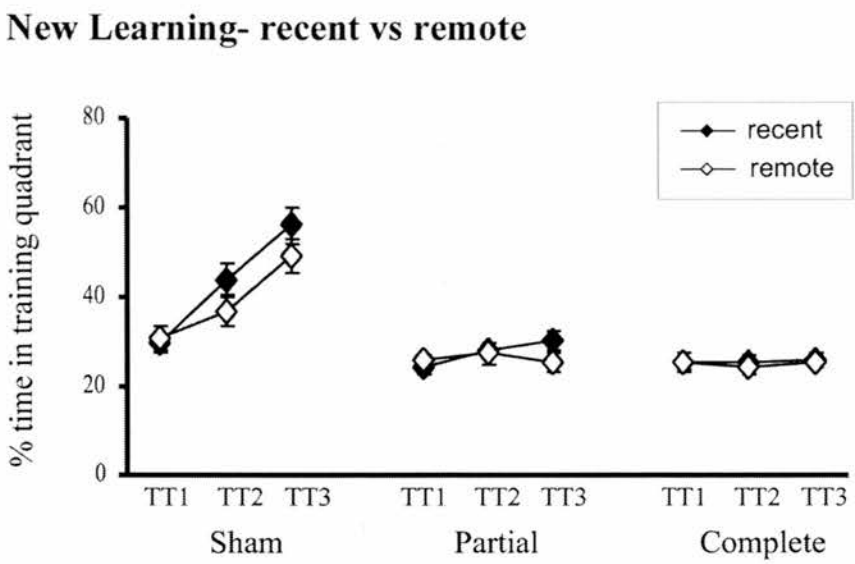


Figure 10.11: New Learning, recent versus remote, % time in training quadrant across transfer tests 1 to 3. For each lesion group the data points correspond, from left to right, to first, second, and third transfer tests respectively.

Figure 10.11 represents % time in target quadrant during the different 60 second transfer tests in both recent and remote time-intervals. An overall analysis revealed an effect of Group ($F [2, 101] = 44.09$, $p < 0.001$), but no effect of Time-interval ($F [1, 101] = 1.85$, $p > 0.05$) and no Group by Time-interval interaction ($F < 1$). It also disclosed an effect of Transfer Test ($F [2, 202] = 27.7$, $p < 0.001$) and a Group by Transfer Test interaction ($F [4, 202] = 20.11$, $p < 0.001$). After analyzing individual groups, the effect of Transfer Test held for the sham group ($F [2, 148] = 12.72$, $p < 0.001$), which showed a patent increase in performance across transfer tests, but not for the partial and complete lesion groups ($F < 1$ for both).

10.3.6 Predictive rats?

Two issues need to be addressed before undergoing discussion of the results presented.

The first issue refers to the volume of hippocampal tissue spared in the recent and remote partial lesion groups. The recent partial group has an average of 31 ± 2%

hippocampus spared ranging between 22 and 50%. The remote partial lesions group, however, has an average of 41 ± 2% hippocampal tissue spared ranging between 28 and 50%. One could argue that this difference in volume does not justify a comparison between the recent and remote conditions. While this is potentially true, I defend the comparison with the following argument. The standard theory of a time-dependent role of the hippocampus in memory suggests that a hippocampal lesion would result in a smaller loss of remote memories compared to recent ones. Having more tissue spared in the remote condition, thus, should facilitate the appearance of such a pattern of results. Despite this, the results suggest a flat gradient of partial retrograde amnesia after partial hippocampal damage. Identical tissue volumes across time-intervals would not, therefore, have changed the key finding and might have revealed, if anything, forgetting with time.

The second issue is more complicated and highly surprising. When analyzing the data, a difference between the behaviour of rats rewarded with the platform in the original quadrant and those rewarded in the novel quadrant was observed already in the first transfer, before the platform was raised in either position. Graphs 10.12.a and b illustrate this effect for the recent and remote conditions respectively. The percentage time in the training, original, quadrant is plotted against percentage time in the opposite quadrant.

Recent- platform effect in TT1

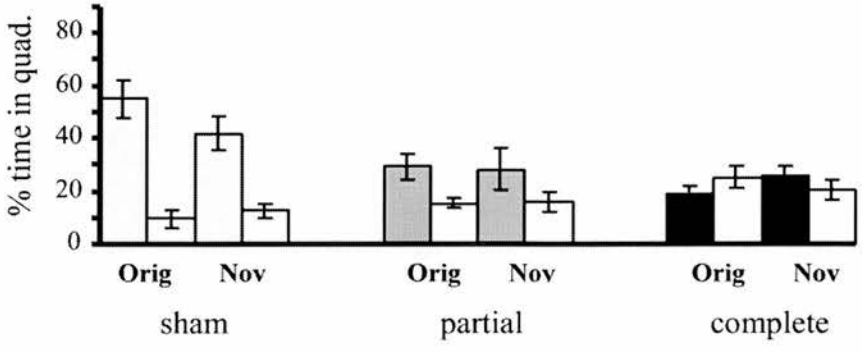


Figure 10.12.a: Recent condition, original versus novel, both plotted as % time in training (coloured bar) and opposite (white bar) quadrants for each group. The platform rests at the bottom of the training quadrant for the original groups and at the opposite quadrant for the novel groups.

Rats that were to be rewarded in the original quadrant are plotted separately from rats that were to be rewarded in the novel quadrant. It becomes apparent that, both in the recent and remote conditions, sham animals show a tendency to spend less time in the original quadrant when they are to be rewarded in the novel quadrant. This effect is less obvious in the partial lesion group, where it appears in the form of more variability in percentage time spent in the training quadrant for rats belonging to the novel group, and is not present in the complete lesion group.

There is only one difference, considering that every other parameter has been counterbalanced carefully, between the original and novel groups at this point in testing: the position of the submerged platform, held at the bottom of the pool to be released at the end of the 60 seconds. Therefore, one has to conclude that the rats can detect the platform. They might detect the field of the magnet used to maintain the platform in the bottom of the pool. Shams and rats with partial lesions in the novel groups, however, show a preference for the original training quadrant, suggesting that this detection of the platform is not sufficient to occlude memory for the training experience. Also, percentage time in the opposite quadrant does not increase in the novel group in comparison to the original group. This suggests that it is not detecting

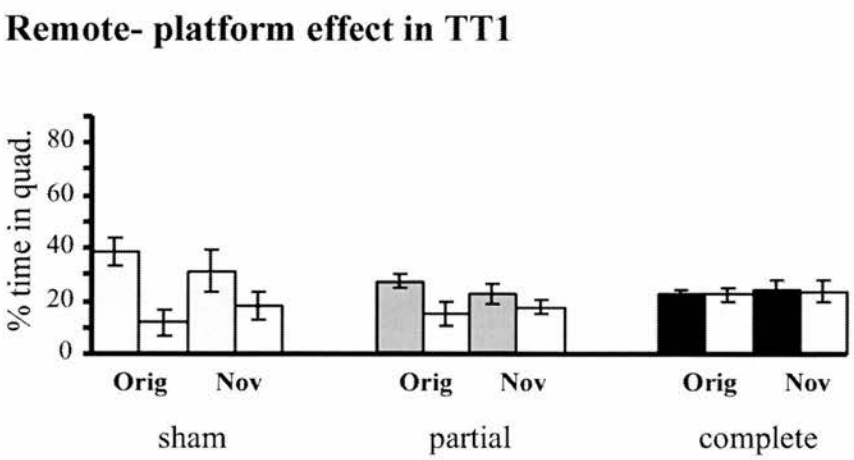


Figure 10.12.b: Remote condition, original versus novel, both plotted as % time in training (coloured bar) and opposite (white bar) quadrants for each group. The platform rests at the bottom of the training quadrant for the original groups and at the opposite quadrant for the novel groups.

the platform over the preferred quadrant, rather, than detecting it over a novel quadrant, that originates the difference between the original and the novel groups.

One can conclude with certainty that the memory observed during transfer test 1 in the sham and partial lesion groups is not the resulting artefact of being able to detect the presence of the platform.

The platform effect, which in the first transfer test in the training quadrant is a mere tendency, is further confirmed when looking at the performance in a novel context. Figure 10.13.a and b illustrates the percentage time in the target and the opposite quadrant during the 60 seconds of the first transfer test. It is apparent that sham animals show a preference for the target quadrant despite the fact that they have never been exposed to that context before. This effect is more severe in the remote time-interval where even rats with partial hippocampal lesions are affected.

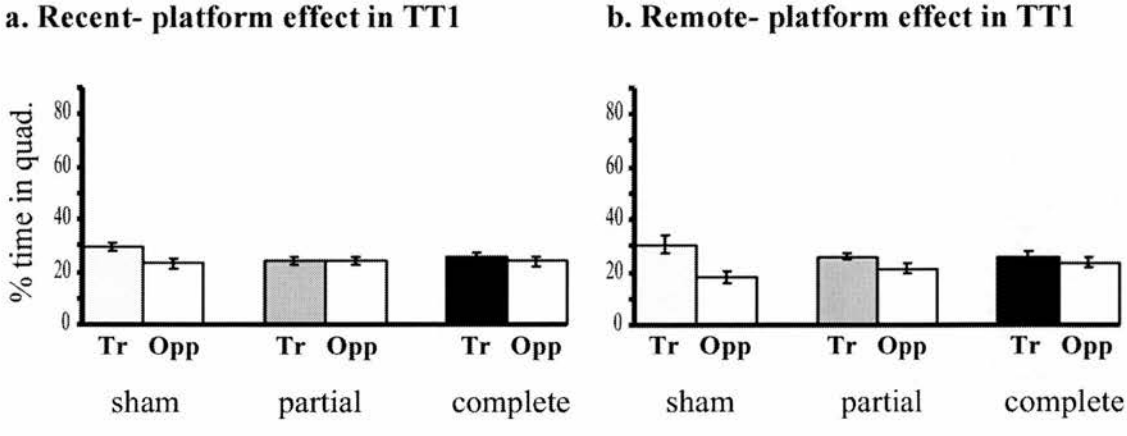


Figure 10.13.a and b: New learning. Recent (a) and remote (b) % time in training (coloured) versus opposite (white) quadrants for the sham, partial and complete lesion groups.

Does this effect of detection of the platform shed doubt over the validity of the results obtained? Although certainly far from ideal, my belief is that the key results obtained in this study are unaffected by this effect. Memory for the original quadrant in the sham and partial lesion groups is observed even in animals that are, at the end of the transfer test, rewarded in a novel quadrant. Detecting the platform in the novel quadrant is, thus, not sufficient to stop them from dwelling in the original quadrant where the platform cannot be detected because it is absent. Also, the fact that rats with complete hippocampal lesions are impaired in the first transfer test and cannot be reminded by exposure to the platform, cannot be affected by detection of the platform. It is possible, however, that results such as the good learning of the novel platform position in shams is influenced by this effect.

The lack of platform effect in the complete lesion group, together with the lack of increased time in the opposite quadrant in the novel groups, would suggest that this effect only appears in combination with memory. New learning, however, is not influenced by memory but it is affected by the platform position in sham and, to a lesser extent, in the partial lesion group. Is this detection, then, hippocampal dependent? Reluctant to accept this possibility I would rather consider that a spatial capacity is necessary in order for the detection of the platform to have an effect on performance.

The following general discussion will be based on those results that I believe to be unaffected by the detection of the submerged platform:

1. Memory for the training platform location in the sham and partial lesion groups and lack of it in the complete lesion group.

2. Absence of improvement across transfer tests (reminding effect) in the complete lesion group.

10.4 Discussion

10.4.1 Time-dependent role of the hippocampus in spatial memory?

Rats that received a complete hippocampal lesion one day or 6 weeks after training did not display a spatial bias during the transfer test performed 2 weeks after the lesions for the area of the pool rewarded with a platform during training.

This result is consistent with previous findings (Bolhuis et al., 1994; Mumby et al., 1999a) but differs from them in the following aspects. Bolhuis et al. (1994) found no clear evidence of memory in the remote sham group, thus, making the interpretation of the deficit found in the complete lesion group difficult. They used a very long, 14 weeks, acquisition to testing interval that allowed for too much forgetting in shams. Mumby et al. (1999a) used a similar interval but got around this forgetting in two ways. First they used a within subject study where training in the recent condition might have helped the memory of shams for the remote condition. Second, the first transfer test was performed on the forth postsurgery trial, thus, having allowed for memory reactivation in the sham lesion group.

The result suggests that the hippocampus plays a time-independent role in spatial memory. It is possible that after 6 weeks a neocortical memory trace is consolidated, but that, in order for this to be retrieved through this new hippocampal-independent route, it needs to be reactivated. With the use of a novel reminding testing protocol, it seems that spatial memory is inaccessible without a hippocampus and that reactivation is not sufficient to trigger hippocampal-independent retrieval.

These results constitute strong evidence that spatial memories, at least those acquired in a reference memory task in the watermaze, are, unlike other types of hippocampal dependent memory, permanently dependent on the structure. The nature of this dependency is unknown. It could, for example, be that the relevant sensory information on the platform location is stored in neocortex but that the hippocampus is required for its retrieval, maybe because, the hippocampus is necessary for the

process of navigation. It is also possible that spatial information is stored in the hippocampus. This issue is further discussed in section 10.5.3.

According to Moscovitch and Nadel's (1998) model of hippocampal dependent memory consolidation, traces are created in a distributed manner within the hippocampus. Thus, the more traces that have been created through pre-traumatic reactivation of the memory, the more likely is that limited damage to the hippocampus will have spared some of those traces. Their prediction, therefore, is that complete hippocampal damage will result in a flat gradient of retrograde amnesia for episodic memory, a type of memory they believe to be ultimately dependent on the hippocampus. In my view, spatial and episodic memory could be underlined by the same process. These two types of memories share a series of characteristics. For example, neither of them can rely on the memory for individual items or set of items. To express these types of memory, it is also not enough to remember one particular contextual setting without being able to place that setting in relation to, or as distinct from, others through time or space. To put it metaphorically, for the expression of both episodic and spatial memory, it is necessary to navigate through time and space respectively. The nature of this process, rather than the particulars of these two types of memories, would then be the reason why spatial and episodic memories are so characteristically dependent on the hippocampus.

A flat gradient of retrograde amnesia for spatial memories is predicted by the cognitive mapping theory of hippocampal function (O'Keefe and Nadel, 1978). The results obtained with complete hippocampal lesioned rats in this study could then be understood to support this theory. While I do not doubt that spatial memory is dependent on the hippocampus, I believe, as argued in the previous paragraph, that this is because spatial processing is underlined by a more general, and hippocampal dependent, type of processing that also subserves episodic memory.

10.4.2 Hippocampal-dependent storage or retrieval?

Why can spatial memories not be reactivated this way? Is it because they are permanently stored in the hippocampus? Is it because, while their storage becomes

eventually hippocampal independent, the structure is always needed for retrieval? Or is it because, although storage and retrieval become with time independent of the hippocampus, the computations required to behaviourally express those memories through navigation are permanently dependent on the hippocampus? At the moment it is not possible to distinguish between these possibilities.

The result obtained from the partial lesion group does not facilitate an answer either. Moser and Moser (1998a) presented data suggesting that during acquisition of a reference memory task in the watermaze the memory trace is distributed along the septotemporal extent of the hippocampus. Rats with approximately 35% (40% and 30% for the remote and recent conditions respectively) of the hippocampus spared performed above chance but worse than sham animals. If these rats had performed as well as shams, it could be argued that consolidation of the trace had made the storage independent of the hippocampus, and that the little hippocampus spared was enough to do the computations necessary for the retrieval of this trace. However, as it is, the limited sparing of memory in the partial lesion group can be attributed to either a distributed memory storage that is only partially affected by the lesion or to limited retrieval or navigational capacity of the volume of tissue spared.

The processing capacity of the hippocampus facilitates acquisition of different types of tasks. Many of them, however, once the appropriate associations have been formed, with the help of the hippocampus, in the form of cortico-cortical associations, as proposed by Squire and Alvarez (1995, described in Chapter 8) can be retrieved in the absence of the structure. Spatial memory, specially a task that involves navigation, might not be possible by simple reactivation of these cortico-cortical associations and might require further processing of these associations during retrieval. The question is whether the activation of these connections between cortex and hippocampus during retrieval are understood as a storage (of the memory for the connection) or as a retrieval process.

Hippocampal lesions have been reported to result in a gradient of retrograde amnesia for radial arm maze reference memory in rats (Cho et al., 1995; Ramos, 1998). The measure of memory in these studies, however, is an average of performance over a

series of rewarded trials. It is impossible in this situation to distinguish between memory and the confounded relearning that might be occurring throughout this testing procedure. One could argue that, was the measure of testing a reliable assessment of memory, the reason why spatial radial arm maze tasks give a temporary gradient of retrograde amnesia while watermaze tasks result in a flat gradient resides in the different demands of these two types of tasks: i.e. the difference in the degree of navigational requirement. This reasoning would support the idea that it is the permanent dependency of navigation on the hippocampus, rather than dependency of storage, that makes the watermaze special.

10.4.3 Temporal hippocampus and memory for a spatial location: implications for human cases of retrograde amnesia

Rats with partial sparing of the hippocampus, namely the temporal 35%, displayed a spatial bias for the area where the platform had been located during training. This was the case for both the recent and remote condition.

In my view the implications of this finding for the traditional understanding of the human literature are enormous. The problem with human cases of amnesia in relation to hippocampal research is that damage in these patients often extends beyond the hippocampus. Thus, when cases are found where damage is limited to the hippocampus, the focus is on the structural selectivity of the damage. More often than not, in these cases, the fact that the damage does not affect the whole extent of the structure is ignored. Here, evidence, is presented that small sparing of the hippocampus can make an important qualitative difference in the outcome of the memory test.

Vargha-Khadem et al. (1997) present behavioural data on three patients with damage limited to the hippocampus. They conclude that spatial, temporal and episodic, but not semantic, memory is impaired as a result of this damage. However, all three patients have some hippocampal sparing that might account for part of the semantic memory capacity. Moreover, Eleanor Maguire (unpublished study) reports that one of these patients, Jon, not only shows some episodic memory sparing and an ability

to distinguish between those events he knows about and those he remembers, but that the remaining of his hippocampus appears active during retrieval in an fMRI study.

Teng and Squire (1999) address the very interesting and relevant question of whether hippocampal damage in a human results in retrograde amnesia for spatial data acquired long time before. They asked this question in a patient, E.P., whose damage extends beyond the hippocampus. In this patient remote spatial memory is intact, suggesting that spatial memory has become hippocampus-independent. This is in clear contrast with the findings reported in this chapter and described in section 10.5.1, that rats with complete hippocampal damage display a flat gradient of retrograde amnesia. However, although damage to the hippocampus in E.P. 'is virtually complete', a small fraction is spared bilaterally. In my view, accompanying the behavioural data with fMRI information is essential in order to ascertain the hippocampal-independent character of the memory reported.

Another aspect of human retrograde amnesia is modelled by the partial lesion group. In the recent condition, the increase in percentage time in training quadrant across transfer tests by the partial lesion group can be interpreted as recession of the retrograde amnesia. The reason to consider the recent, but not the remote, partial lesion group is that the recent, but not the remote, 'original' partial lesion group is different from the 'novel' counterpart. Recession of retrograde amnesia is often observed in amnesic patients (Tulving et al., 1988) in the form of a decrease of the severity of the memory loss. The patient H.M., for example, was reported to have retrograde amnesia covering the 15 years previous to the onset in the first report (Scoville and Milner, 1957) but covering only 2 years in a later follow up study (Milner et al., 1968). After the onset of retrograde amnesia these patients are submitted to numerous memory tests and are, themselves, likely to go over what they remember of their past, contrasting it with their relative's knowledge of it. The reminder protocol that has been used in the study presented in this chapter could be understood to be similar to this process of attempting to recover memory in amnesic patients.

Moscovitch and Nadel (1998) are an exception among the theorists of memory consolidation in acknowledging the importance the differential hippocampal sparing might have on the variability of the amnesia phenotypes among human amnesics. In their model, reactivation of memories through time is the key cause of the appearance of a process of consolidation. As discussed in the previous chapter, I am uncertain as to what exactly they refer to by reactivation of the memory. The results obtained from the partial lesion group suggest that this reactivation must be driven by experience. Rats with partial damage to the hippocampus show a flat (not graded), but partial, retrograde amnesia. If reactivation occurred through time and not necessarily in the presence of the relevant cues, a gradient of retrograde amnesia should have been observed in this group of animals. A flat gradient of partial amnesia could be explained by the fact that both the recent and remote groups have had the same amount of experience dependent reactivations (an identical number of trials in the pool).

Focusing now on the recent partial lesion group, the observed limited sparing of memory contrasts with the findings by Moser and Moser (1998a). In this watermaze study, rats received lesions to the septal or temporal parts of the hippocampus immediately after a transfer test performed the day after the end of training (6 days). Only rats with the septal 2/3 of the hippocampus spared demonstrated memory for the location of the platform. Here rats with the temporal 35% of the hippocampus spared show memory. It is possible that the memory measurement used in the study by Moser and Moser (1998a), percentage time in a circular area around the platform position, is too strict to reveal the limited memory observed in the recent partial lesion group.

Finally, I want to emphasise the fact that rats with only the temporal 35% of the hippocampus spared display spatial memory. This result supports the findings presented in Part 1 of this thesis that both septal and temporal areas of the hippocampus are involved in spatial learning and can independently support learning of a reference memory task in the watermaze.

10.4.4 What next?

Results presented in Chapter 9 suggest that there is a process of consolidation that is independent of retrieval and takes place in the hippocampus. Evidence for this is found in the deficit caused by inactivation of the hippocampus during the interval between acquisition and testing. According to the data this process is still ongoing at least 5 days (time of the latest LY minipump implantation) after acquisition. Inactivation during retrieval also resulted in an impairment in performance. However, this latter deficit could be dependent on the fact that the process of retrieval-independent consolidation is still ongoing.

To what extent are the hippocampal-dependent process of consolidation (as independent of retrieval) and the hippocampal-dependent process of retrieval linked? Do these two process evolve together such that they become independent of the hippocampus in parallel? Or is retrieval dependent on the hippocampus only for as long as the process of consolidation is still ongoing in this structure?

This question is related with the one posed in section 10.5.3 of whether the flat gradient of retrograde amnesia observed for watermaze consolidation studies is the result of a hippocampal-dependent storage or of a hippocampal dependent retrieval of these memories.

A way to further explore this issue is by using an experimental design identical to that described in Chapter 9 but with the longer (8 weeks) interval between acquisition and testing used in this chapter. Two groups would be tested, those with LY minipumps implanted 6 weeks after acquisition (LY would wash out aprox. a week before retrieval) and those with LY acutely infused just before retrieval. Appropriate aCSF controls would be used. If inactivation during the days previous to retrieval resulted in no deficit, but inactivation during retrieval did, one could conclude that the two mentioned processes (consolidation and retrieval) are independent and that hippocampal dependent consolidation is terminated. This result would also indicate that the reason why watermaze studies of memory consolidation

consistently find a flat gradient of retrograde amnesia is because the hippocampus is permanently necessary for retrieval of spatial memories in this task.

10.4.5 Conclusions

Post-training lesions of the whole of the hippocampus in rats result in a flat gradient of retrograde amnesia for a reference memory task in the watermaze. The memory for the location of the platform cannot be reactivated by repetitive exposure to the platform suggesting that this memory does not become supported by the neocortex with time and that retrieval or storage of the memory are permanently dependent on the hippocampus.

Rats with the temporal 35% of the hippocampus spared, on the other hand, show spatial memory, although this is quantitatively identical whether the lesion was made one day or 6 weeks after training. This result supports the findings in Part 1 of this thesis that the temporal hippocampus is involved in spatial memory. It also highlights the importance hippocampal sparing might have on human cases of retrograde amnesia where hippocampal damage varies in severity and is seldom complete.

Sham animals showed forgetting over time, a phenomenon that is consistently observed in both human and non-human studies of memory consolidation.

Chapter 11

General Conclusions

Chapter 11

General Conclusions

This thesis set out to explore two fascinating and, as yet, not well known aspects of hippocampal function: the possibility that the structure is functionally differentiated along its longitudinal axis and its role in memory consolidation with particular focus on spatial memory.

The majority of the work presented in this thesis is based on the behaviour of rats with partial and complete ibotenic acid hippocampal lesions. Chapter 3 presents a thorough assessment of these lesions. Using retrograde tracer techniques, the spared tissue is shown to maintain the normal major inputs. The method to determine the amount of hippocampus spared is compared with methods used by others and concluded to be the most reliable. Its pitfalls are carefully explored and determined as minor.

Part 1 of the thesis addresses the question of whether the hippocampus is divided in functional units along the septotemporal axis, an issue of enormous implications for the controversial field of hippocampal function.

Results obtained further in the thesis made it necessary to begin by establishing the replicability of the findings by Moser and colleagues (1993, 1995), that lesions to the septal, but not lesions to the temporal, hippocampus impair spatial memory of a reference memory task in the watermaze. It is, thus, established that the results can be replicated: volumes of hippocampus varying between as little as 20 to 40% of the structure can support spatial memory in this task, provided the tissue is located septally. This suggests that hippocampal encoding of space is secluded to the septal pole of the hippocampus and, even more, that it is not topographic.

In Chapter 6, the consequences of this finding are further explored. By assessing the behaviour of rats with different volumes of septal bilateral and unilateral sparing, it is concluded that memory for the spatial reference memory task can also be achieved with unilateral septal tissue and that this can be as little as 30% (15% of the total hippocampus). These findings suggest that the reference memory task is not as sensitive a test of hippocampal function as it is traditionally believed. Moreover they suggest that the assessment of drug treatments affecting a small region of the hippocampus is not best performed by using this type of task. These findings, however, also suggest a solution to this problem: lesions to most of the structure leave performance in the reference memory task unaffected but provide a means of making the localized effect of a drug reach the totality of the hippocampus spared. The behaviour of the same rats in a delayed-matching to place task suggests that such small volumes of tissue are not sufficient to support learning of a spatial task that requires behavioural flexibility. Even though the task is performed in the same watermaze as the reference memory task, none of the lesioned groups can hold memory for the daily platform position for as long as 20 minutes, although all groups show a degree of memory after 5 seconds. Thus, when the demands of the task increase, the required volume of hippocampus also increases. As the environmental and procedural aspects of both tasks used are identical, it is suggested that hippocampal tissue encodes not only for spatial but also for behavioural aspects of the task.

In Chapter 7, the attention turns to the temporal hippocampus and whether the observed incapacity of this part of the hippocampus to support learning of the reference memory task can be generalized to all forms of spatial memory. Surprisingly, rats with as little as 20-40% of the hippocampus spared temporally can learn a reference memory task in one and, even, two concurrent watermazes at the same rate as the equivalent septal spared group. These results constitute evidence towards a unitary hypothesis of hippocampal function. The difference between these results and those of Moser et al. (1995) strives from subtle modifications of the training protocol: spacing of trial sessions and higher number of days of training, but a smaller total number of trials. It cannot be drawn from the data why rats with septal hippocampus spared are favored by a high trials/day ratio while rats with temporal

hippocampus spared do not seem to react to the training until the 6th day. In relation to this issue, differences in the intrinsic and extrinsic circuits along the longitudinal axis, as well as possible differences in the effect of the lesion, are discussed.

The finding that rats with only temporal hippocampus spared can acquire a spatial task opens very interesting questions. The functional differentiation hypothesis drew attention towards the segregation of projections along the septotemporal axis. In my view, implying a functional differentiation from this segregation is denying the hippocampus its more interesting role, that of finding associations across inputs of all modalities. My believe is that, a more interesting question refers to the process by which this segregation contributes to the function of the hippocampus and the role played by the longitudinal intrinsic connections that run across the limits established by the levels of termination of the extrinsic projections.

Part 2 of the thesis explores the difficult subject of hippocampal dependent memory consolidation, particularly that of spatial memories, by capitalizing on the novel possibility of temporarily inactivating the hippocampus, and also by the use of a novel testing protocol.

Part 2 begins by assessing the involvement of the hippocampus in different stages of memory by temporarily inactivating hipocampal AMPA receptors during acquisition, retrieval or the training to testing interval (consolidation period) of a reference memory task in the watermaze. Impairments are observed as a result of inactivations during each memory stage suggesting an involvement of the hippocampus in each of them. That inactivation of the structure between acquisition and retrieval can also cause an impairment during testing, suggests that there is a hippocampus-dependent consolidation period that is independent of retrieval. This is a very interesting result, as this cannot be directly implied from human cases of retrograde amnesia.

The deficit resulting from inactivation during the consolidation period suggest that the hippocampus-dependent consolidation process takes longer than the short training to testing interval (2 weeks) used in this study. The deficit observed when the rat is tested with an inactivated hippocampus could, thus, be dependent on the consolidation period still taking place. It might be possible that, once this process is

completed, retrieval then becomes also hippocampal independent, as is suggested by human cases of graded retrograde amnesia.

Chapter 10 explores whether retrieval of spatial memories can occur in the absence of the hippocampus when sufficient time is given for the memory to consolidate. Previous studies suggest that this is not the case but use a traditional single transfer test. In this study, the possibility that neocortical traces of spatial memories need to be reactivated is taken into account and tested with the use of a reminder protocol. Lesions to the hippocampus given six weeks after training, however, result in chance performance during the first transfer test. Moreover, no improvement was observed after 2 reminders suggesting that the hippocampus is permanently required for the expression of spatial memories. Whether this is because the memory is not consolidated, or because the hippocampus is necessary for retrieval is not known. However, the fact that this flat gradient of retrograde amnesia is only observed in tasks that require navigation suggests that the hippocampus is always required to perform this navigation.

In the same study, rats with the temporal 35% of the hippocampus spared displayed a spatial bias, although limited. This was true of both animals lesioned one day or 6 weeks after training. Partial hippocampus sparing is often seen in human cases of amnesia, nonetheless, the memory capacities of these patients are often attributed to a hippocampal independent process. The result presented here suggests that little hippocampus sparing can account for more than it is generally expected. Thus, imaging studies should accompany the memory assessment of humans with partial damage to the hippocampus.

Although Part 1 and Part 2 of this thesis set out to explore two orthogonal subjects, the results from both sections converge in an interesting manner. Findings throughout this thesis suggest that with little hippocampus sparing (septal or temporal) animals can not only learn but also express memories acquired before the lesion. Moreover, the results from the partial lesioned group in Part 2 support the novel finding, in Part 1, that the temporal hippocampus has the capacity to process spatial memories.

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Reversible neural inactivation reveals hippocampal participation in several memory processes

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Studies of patients and animals with brain lesions have implicated the hippocampal formation in spatial, declarative/relational and episodic types of memory. These and other types of memory consist of a series of interdependent but potentially dissociable memory processes—encoding, storage, consolidation and retrieval. To identify whether hippocampal activity contributes to these processes independently, we used a novel method of inactivating synaptic transmission using a water-soluble antagonist of AMPA/kainate glutamate receptors. Once calibrated using electrophysiological and two-deoxyglucose techniques *in vivo*, drug or vehicle was infused chronically or acutely into the dorsal hippocampus of rats at appropriate times during or after training in a water maze. Our findings indicate that hippocampal neural activity is necessary for both encoding and retrieval of spatial memory and for either trace consolidation or long-term storage.

The study of brain dysfunction caused by lesions has revealed evidence that the hippocampal formation and related neocortical structures are involved in various 'types' of memory[1–5]. A logically separate issue concerns the participation of this (or other brain areas) in distinct memory 'processes'—namely encoding, retrieval, storage and consolidation[2,6–9]. Permanent lesions cannot unambiguously dissociate potentially separable memory processes. Lesions made before training would impair later recall were they to disrupt any of these four memory processes; those made after training could, depending on their time of administration, cause deleterious effects by disrupting the integrity of trace storage, time-dependent consolidation processes or retrieval. Reversible temporary inactivation offers an opportunity to isolate the obligatory contribution of hippocampal activity to these processes.

The advantage of using reversible local inactivation is that it should disrupt any memory-related process for which normal neural activity in a brain structure is required, without affecting other memory-related processes engaged at later times[10]. First, with respect to encoding-related and retrieval-related memory processes, we reasoned that chronic inactivation throughout training and acute inactivation during a single retention trial should each be sufficient to disrupt these memory processes independently. We therefore used both drug infusion techniques successively in individual animals. Second, with respect to consolidation processes, we made the reasonable guess that chronic inactivation for seven days, beginning one day after the end of training, might be sufficient to shut down a long-term consolidation process[2]. It is unlikely that neural inactivation beginning one day after several days of training would affect short-term consolidation mechanisms, an assumption tested with relevant control groups. Third, we also recognised the possibility that chronic hippocampal inactivation after training might disrupt the maintenance of altered synaptic weights, widely assumed to underlie information storage in the hippocampus[11,12].

Reversible inactivation of the hippocampus was achieved by blocking fast glutamatergic synaptic transmission using the selective AMPA/kainate receptor antagonist LY326325 (ref. 13). Electrophysiological and two-deoxyglucose methods were first used to validate this method of neural inactivation with respect to its magnitude, time-course and regional extent. We then examined hippocampal participation in these four memory processes using a modified reference memory water maze task designed to maximize the persistence of spatial memory.

Results

Reversible neural inactivation of the hippocampus

LY326325 is a different salt of the better known decahydroisoquinoline compound LY293558 (refs. 13, 14) that blocks the glutamate receptor subunits GluR1–5. It was chosen because its excellent water solubility enabled artificial cerebrospinal fluid to be used as a vehicle for continuous infusion of the hippocampus for up to 14 days. Thus we could avoid the brain damage that would occur with organic solvents such as DMSO, which are required for the initial solubilization of quinoxalinedione AMPA receptor antago-

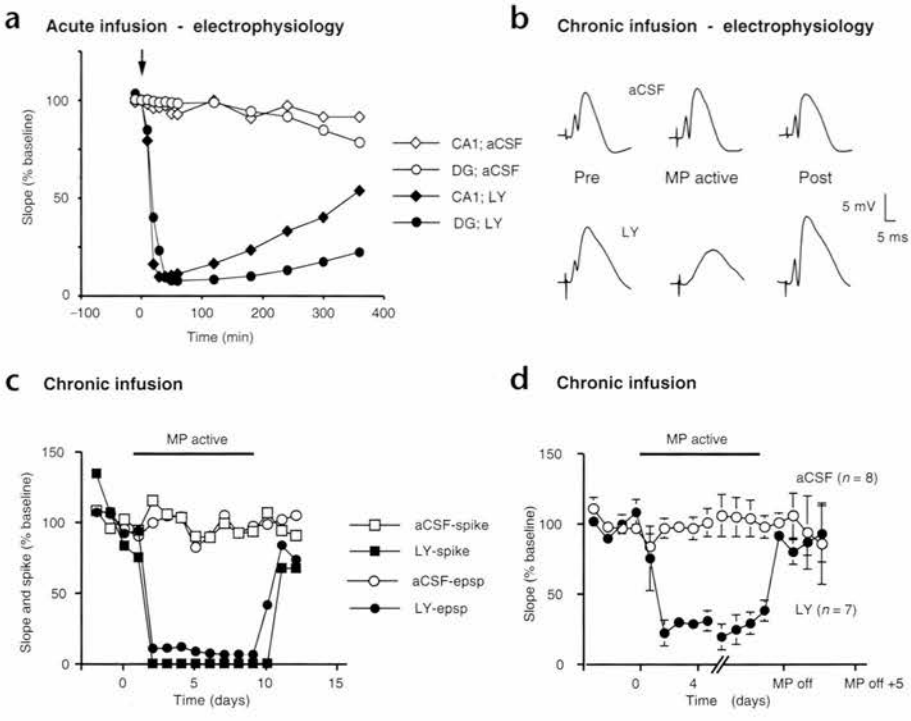

Fig. 1. The AMPA/kainate receptor antagonist LY326325 blocks fast synaptic transmission at perforant path/granule cell synapses. (**a**) Acute field potential recordings in urethane-anesthetized rats in response to stimulation at 0.05 Hz. Filled symbols, LY-treated rats; open symbols, vehicle-treated rats (aCSF). Dentate potentials in response to perforant path stimulation (circles, $n = 6, 6$); CA1 potentials in response to homotopic stimulation of the contralateral hippocampus (diamonds, $n = 8, 8$). Data are normalized to a stable baseline (20 min, two data points) and pooled over 10-min periods. Intrahippocampal infusion (arrow) of LY (1 µl, 1.5 mM, over 10 min) caused a decrease in field-potential slope of about 90%, which was maintained with only gradual recovery over 6 hours; vehicle had no effect on baseline signals. (**b**) Chronic recordings from representative freely moving rats. Recording 1 ('Pre') was obtained during baseline (when all animals had continuous vehicle treatment). Recording 2 ('MP active', that is, under LY) shows the reduction in the early rising slope of the extracellular field potential and lack of population spikes. The nature of the slow residual signal remains unknown. Recording 3 ('Post') shows full recovery of potentials after cessation of the micropumps. (**c**) Full time course of representative vehicle and LY micropump-implanted animals. Means for dentate slope and population spike. (**d**) Pooled data from chronic dentate recording experiments (vehicle, $n = 8$; LY, $n = 7$; mean ± s.e.). Data were averaged both forward (locked to time to when micropump was changed) and backward (locked to moment when within 20% of normal field-potential was restored). The use of backward averaging was needed to show the abrupt return to normal in all animals when the micropump stopped pumping. (Duration of pumping could vary by ± 1 day.)

nists such as CNQX[15]. We chose a glutamate receptor antagonist over a local anesthetic (such as lidocaine) to avoid disrupting fibers of passage through the dorsal hippocampus. This is analogous to making a neurotoxic rather than an aspiration or electrolytic lesion[16], with the added advantage of reversibility. As this single drug was used throughout, we shall hereafter refer to it as 'LY'.

The extent and time course of temporary inactivation of the hippocampus was examined electrophysiologically in rats. In acute experiments, we monitored either dentate gyrus field potentials in response to perforant path stimulation, or CA1 potentials in response to stimulation of the homotopic contralateral CA1 region. Infusions of LY (1 µl, 1.5 mM) decreased extracellular field potentials by up to 90% with a very gradual recovery over 4–6 hours (**Fig. 1a**). Vehicle infusions had no effect. Dentate population spikes disappeared completely; CA1 stimulation was always below spike threshold.

Chronic LY experiments in awake animals with previously implanted electrodes and intrahippocampal cannulae were then

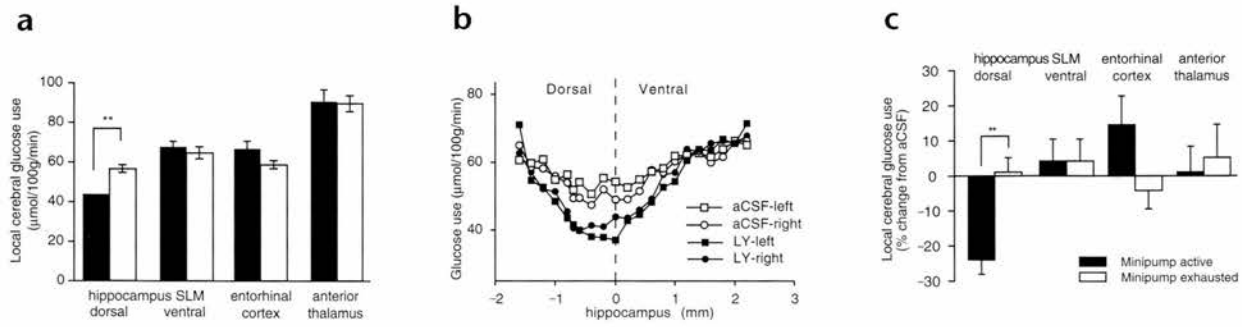

Fig. 2. The AMPA/kainate receptor antagonist LY326325 reduces glucose utilization in the dorsal hippocampus. (**a**) Absolute levels of local cerebral glucose use in 4 memory-related brain areas 4 days after the start of chronic infusion into the dorsal hippocampus. Black bars 0.375 mM LY at 0.5 µl per h, $n = 5$; vehicle, $n = 5$; **p=0.002, Student's t-test. (**b**) Local cerebral glucose use in the molecular layer of CA1 along the full longitudinal axis of the hippocampus of both hemispheres. (**c**) Normalized data for relative glucose utilization by vehicle and LY groups when the micropump was active (4 days after start of infusion, as in **a** and **b**) or exhausted (11 days; vehicle, $n = 4$; LY, $n = 5$). In this panel only, black and white bars reflect times of glucose measurement after micropump implantation.

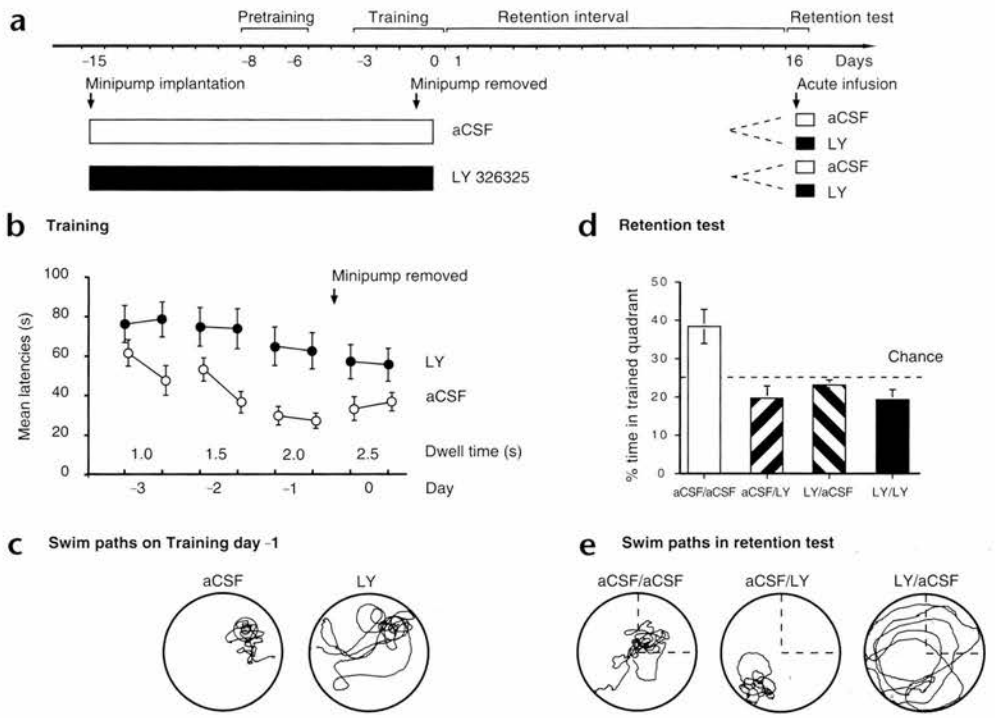

Fig. 3. Hippocampal inactivation disrupts encoding- and retrieval-related processes of spatial memory. (**a**) Experimental design showing chronic infusion of vehicle (open panel) or LY (black) over 14 days, followed after 16 days, by acute infusions of either vehicle or LY. Day 1 was always the first day of the retention period after the end of training. Training was conducted over 4 days (day −3 to day 0), with the 14 day minipump removed on day −1 (see Methods). (**b**) Escape latencies of groups trained during continuous chronic infusions of vehicle or LY (vehicle, $n = 13$; LY, $n = 11$; mean ± s.e.; $F_{1,22} = 9.31$, $p < 0.01$). (**c**) Representative paths taken by a vehicle- and an LY-treated rat on day −1 of training. Note the direct path of the control animal and efficient search at the correct location; the path of the LY-treated animal is more circuitous. (**d**) Proportion of time spent during the 60-s retention test in the 'correct' quadrant of the pool that had formerly contained the escape platform. The groups differed significantly (mean ± s.e.; $F_{3,20} = 8.04$, $p < 0.001$). Subsequent comparisons using Dunnett's test showed the vehicle/vehicle group was significantly better than the other three groups, which did not differ from one another ($p < 0.05$). (**e**) Representative paths taken during memory recall by a vehicle/vehicle, LY/vehicle and vehicle/LY rat. Note that the animal trained under vehicle but tested under LY shows focused search, but at an inappropriate location in the pool, whereas the animal trained under LY but tested under vehicle fails to show a focused searching strategy (dotted line within pool, training quadrant).

used to determine the time course of temporary inactivation and recovery over several days. Stable baselines were first obtained during continuous unilateral infusion of vehicle from a 14-day osmotic minipump. After 10–12 days, this was replaced with a 7-day micropump containing 0.375 mM LY or vehicle, and field potentials were monitored daily. A representative LY-treated rat (**Fig. 1c**) showing both field-potential slope and population spikes reveals that dentate field potentials 'switched off' over one day and then 'switched on' again seven to eight days later. As the exact moment of switching on varied from animal to animal, averages were obtained by aligning data both with respect to when the micropump containing LY was implanted and separately with respect to the point at which field potentials returned to within 20% of normal. The resulting 'split plot' reveals the abrupt decrease in fast synaptic transmission, which remained inhibited throughout the infusion period (with some animals showing a slow late potential; **Fig. 1b**) and then the relatively abrupt return to baseline levels in individual animals within one day of micropump exhaustion (**Fig. 1d**). Signals in vehicle controls remained at baseline throughout.

The spatial distribution of the effect of intrahippocampal infusion was examined by measuring function-related glucose use with [^{14}C]2-deoxyglucose autoradiography (2-DG)[17]. Peripheral administration of LY293558 reduces glucose utilization throughout the brain[18], but more localized changes should occur during chronic intracerebral infusion. Animals were implanted with bilateral cannulae into the hippocampus connected to seven-day micropumps and examined with standard 2-DG techniques after either 4 days (during drug infusion) or 11 days (after micropumps were exhausted).

At 4 days, local cerebral glucose utilization was reduced by 23% in the stratum lacunosum moleculare of the dorsal but not ventral hippocampus (**Fig. 2a**). No changes were observed in other memory-related areas (entorhinal cortex, anterior thalamus) or in over 20 other brain areas examined (data not shown). The maximal reduction in glucose use occurred along the dorsal part of the longitudinal axis adjacent to the implant site (**Fig. 2b**), with an equivalent decrease in both hemispheres. At 11 days (micropump exhausted), glucose use returned to levels indistinguishable from those of vehicle-treated animals (**Fig. 2c**). Thus, intrahippocampal infusion of LY reversibly decreases fast synaptic transmission in a spatially restricted manner so that dentate granule cells no longer fire action potentials for at least seven days but function in an apparently normal manner thereafter.

Memory encoding and retrieval

To examine whether reversible hippocampal inactivation could affect the encoding or retrieval of memory, we treated animals with vehicle or LY during training and/or during retention using a 2 × 2 factorial design (**Fig. 3a**). We disrupted the dorsal hippocampus during training (to determine if this affected encoding-related memory processes) and disrupted it during a later retention test (to determine if this affected retrieval). These manipulations were done symmetrically, which would not be possible with permanent lesions.

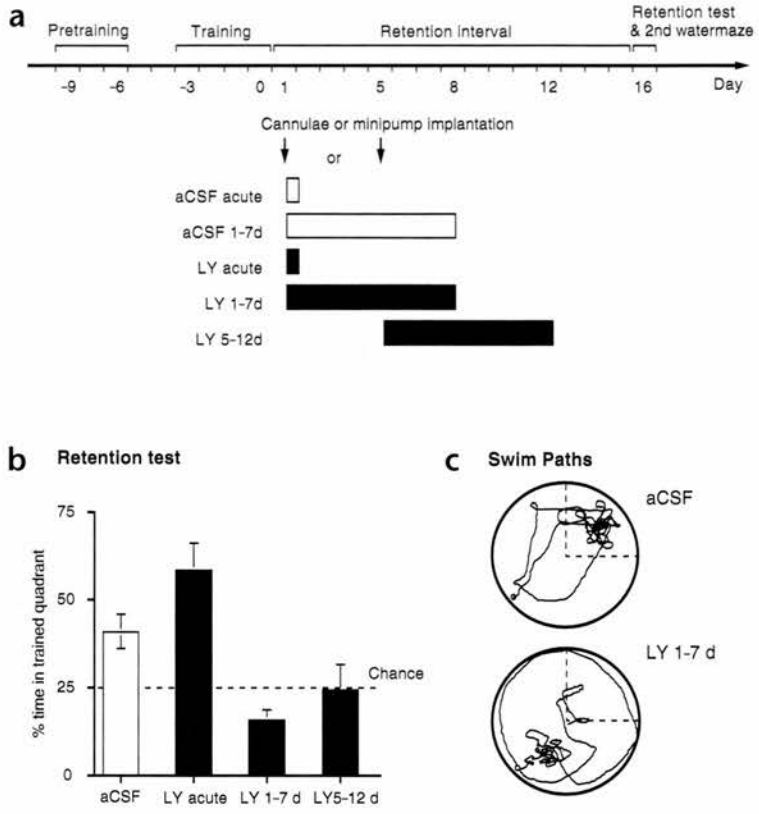

Fig. 4. Chronic but not acute inactivation of the dorsal hippocampus interferes with a post-training memory process. (**a**) Experimental design shows spatial training by normal animals followed by the five different treatments beginning one or five days later. The retention test was always conducted 16 days after the end of training. (**b**) Retention test. The two vehicle control groups did not differ and were combined into a single group ($n = 17$). The 5 groups differed overall (means ± s.e.; $F_{4,43} = 7.76$, $p < 0.0001$). Subsequent comparisons using Dunnett's test showed the two groups treated with LY for 1–7 days ($n = 8$) and 5–12 days post-training ($n = 8$) were impaired relative to the vehicle group ($d = 2.30$, $p < 0.05$). The vehicle-treated group showed unexpectedly poorer recall than the group treated acutely with LY ($n = 8$) at 1 day after training ($d = 2.32$, $p < 0.05$). (**c**) Representative paths taken by the animals treated for seven days after training with vehicle or with LY. Note that both animals were trained before infusion, and the retrieval test was conducted after the micropumps were exhausted. Only the vehicle animal searched preferentially in the training quadrant (dotted line).

Rats were trained on a variant of the open-field water maze in which the escape platform rises to within 1.5 cm of the surface of the water if and only if the animal is both accurate (< 20 cm) and persistent (increasing from 0.5 to 2.5 s) in searching at the correct location[19]. An automated tracking system, calculating the animal's location in real time, triggered the release of the 'Atlantis' platform at appropriate times. Following pretraining and 4 days of spatial training, retention was tested after a 16-day interval.

During training, chronic LY caused a clear deficit in spatial performance (**Fig. 3b**). Paths taken on the third day of spatial training (Day –1, just before the micropumps were disconnected; Methods) showed a representative control rat taking a direct path to the platform area and then remaining there until the hidden platform rose. The LY-treated animal was more circuitous (**Fig. 3c**). The issue is to establish whether this impaired performance is a memory deficit, with memory subdivided into encoding-related and retrieval-related processes. To address this, we subdivided the vehicle and LY training groups before the retention test. Half the animals in each group were given acute intrahippocampal infusions of drug or vehicle (1.5 mM LY or vehicle, 1 µl over 5 min, 1 h before testing). Animals treated with vehicle during both training and retention performed well, whereas those treated with LY in both phases performed poorly (**Fig. 3d** and **e**). The new findings made possible by the use of reversible inactivation are from the group treated with LY during training but tested under vehicle (enabling any effect of hippocampal inactivation on encoding-related processes to be identified) and from the group treated with vehicle during training but tested under LY (enabling a role in memory retrieval to be identified). Both these groups performed at chance. An analysis of variance showed that the four groups differed overall, with planned comparisons revealing the vehicle/vehicle subgroup to be significantly better than each of the other three during memory recall ($p < 0.01$).

The paths taken during the retention test (**Fig. 3d**) differed among groups. Rats trained with LY but tested with vehicle tended to swim all over the pool. However, rats trained with vehicle but tested with LY showed the appropriate searching strategy but failed to execute it in an appropriate location. This was analyzed by computing the maximal time spent in any quadrant of the pool. The mean percentage times for the vehicle/vehicle, vehicle/LY and LY/vehicle groups respectively were 40.9 ± 2.9%, 42.4 ± 3.1% and 31.6 ± 0.9% ($F_{2,16} = 4.27$, $p < 0.05$). Subsequent orthogonal comparisons showed that maximal quadrant time in the vehicle/vehicle group did not differ from that in the vehicle/LY group ($F < 1$), whereas the mean for these two groups was higher than that of the LY/vehicle group ($F_{1,16} = 9.39$, $p < 0.01$). This dissociation suggests the localized searching strategy is not acquired during hippocampal inactivation, perhaps because the animal cannot encode where the platform is located[20], but that it can be performed during retrieval, provided it has been learned earlier, even if the animal cannot remember where to search.

Trace storage or long-term consolidation

We then examined whether temporary hippocampal inactivation could disrupt processes occurring after training but before recall. Various treatments immediately after training (for example, electroconvulsive shock, drugs, brain stimulation) cause deficits or enhancements of several types of memory tasks[21–24]. These are thought to affect a short-term consolidation process that is vital for encoding traces into long-term memory. We focused on a longer-term consolidation process, in which the hippocampal formation and other structures of the medial temporal lobe are implicated[2,25]. Long-term consolidation is thought to involve the interaction of hippocampal and neocortical networks and so require fast synaptic transmission.

Unlike the previous experiment, in which the training and retention phases were conducted under various combinations of drug or vehicle, training in this experiment occurred before treatment, and the memory retention test occurred after micropump exhaustion, with the hippocampus again working normally. Acute or chronic inactivation happened in between. One day after the end of spatial training, the animals were divided into five groups matched with respect to their training performance (**Fig. 4a**). Two

groups were given intrahippocampal cannulae for acute LY or vehicle infusions administered four hours later, two received bilateral seven-day micropumps infusing into the dorsal hippocampal formation, and the fifth group was left undisturbed until five days later, when they were also implanted with bilateral seven-day micropumps containing LY. An additional caudate-infusion group was also trained, but its results are considered separately (below). The retention test was conducted 16 days after the end of training in all groups.

The results showed a highly significant difference in retention between groups (**Fig. 4b** and **c**). The two vehicle control groups did not differ and were combined. These controls remembered the former location of the platform well, swimming repeatedly over the correct location. In contrast, groups given seven-day infusions of LY into the dorsal hippocampus, whether beginning one or five days after training, showed poor memory recall. Once again, the LY-treated animals showed a tendency to remember the strategy of localized searching, albeit at an inappropriate location. Animals given acute infusions of LY in which the duration of hippocampal inactivation is short-lived (**Fig. 1a**) showed, if anything, a trend toward better memory than controls. These findings suggest that the integrity of normal neural activity in the hippocampal formation is necessary for later retrieval for at least five days after training.

Memory-process specificity

The memory-process specificity of this deficit in storage or long-term consolidation was explored in several ways. The first step was to check that it was indeed independent of any effect of LY on retrieval (encoding-related processes being necessarily excluded because the animals had been normal during training). To do this, the learning and retention of a second water maze task was tested on the same day that retention had been tested in the previous experiment (using the same animals). We reasoned that the encoding and retrieval phases of this additional task should be conducted as quickly as possible after the previous experiment because any residual hippocampal dysfunction caused by seven days of temporary inactivation might be transient. Accordingly, immediately after the retention test (Day 16, **Fig. 4a**), all five groups were given six trials of training in a second water maze in a separate room and then tested for retention five hours later (that is, within the domain of long-term memory, but not requiring long-term consolidation). All groups learned rapidly at the same rate (**Fig. 5a**) and did not differ in their memory of the platform location five hours later (**Fig. 5b**). Thus, if there is any 'residual hippocampal dysfunction' after temporary inactivation, it does not affect encoding or retrieval. The basis of the deficit in the previous experiment must be in some other memory-related process.

Two other specificity tests were conducted. First, we double-checked whether chronic intrahippocampal infusion could be causing residual hippocampal dysfunction in encoding or retrieval-related processes. Two new groups of animals were given chronic infusions beginning and ending before the start of all training. Starting 9 days after the micropumps were exhausted, the groups that had been treated with LY or vehicle showed equivalent rates of learning (data not shown) and good recall 16 days later (**Fig. 6a**). Second, anatomical specificity was examined using the same consolidation design (**Fig. 4a**), but with chronic bilateral LY infusion into the caudate. (This group was, in practice, trained at the same time as those infused into the hippocampus, but it addresses a logically distinct issue and is therefore considered separately.) Caudate-treated animals showed good memory recall, performing significantly better than the group infused with LY into the dorsal hippocampus over the same time period and no differently from vehicle controls (**Fig. 6b**). Thus, chronic inactivation of the dorsal hippocampus but not the caudate after training can impair retention of hippocampal-dependent spatial memory by disrupting a memory process other than encoding or retrieval.

Discussion

These findings imply an obligatory but independent participation of hippocampal neural activity in encoding- and retrieval-related processes of one type of memory—spatial memory—and its participation in either long-term consolidation or storage processes. We consider our method of inactivation and these memory processes in turn.

Reversible inactivation of neural activity

Our use of both acute and chronic infusion of a selective glutamatergic antagonist revealed that it is possible to 'switch off' the dorsal hippocampus for varying periods, and for it then to 'switch on' again and apparently work normally thereafter. The electrophysiological data showed residual low-amplitude field potentials during LY infusion but no population spikes; evoked potentials returned when the micropumps were exhausted. The return to near-normal levels is intriguing, as homeostatic changes in AMPA receptor number and efficacy may occur during inactivation. In culture, long-term AMPA receptor inactivation causes a reversible, quantitatively scaled increase in synaptic strength[26,27]. If this reversible homeostatic plasticity were also to occur *in vivo* during chronic infusion, resumption of baseline field potentials would be expected. The 2-DG data revealed an anatomically specific decrease in function that also returned to normal after infusion. Dynamic

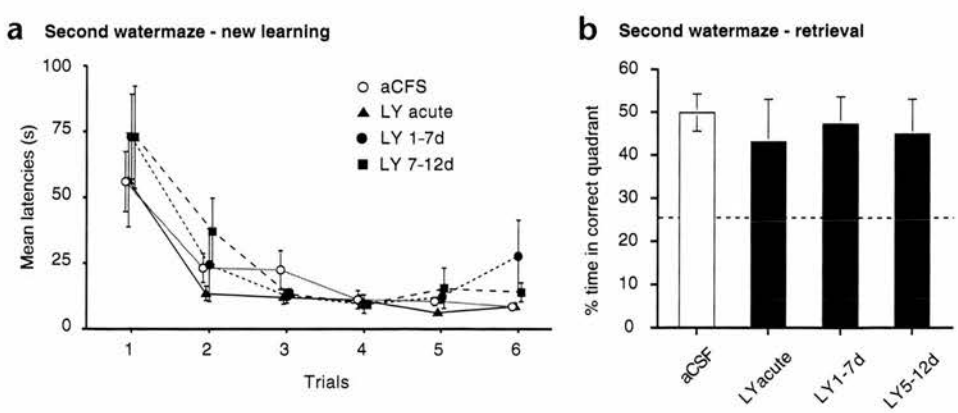

Fig. 5. Chronic LY interferes with a post-training memory process but does not cause any residual impairment of encoding or retrieval. (**a**) Animals that had completed the previous experiment (**Fig. 4**) were also trained in a second water maze in a different room using a standard reference memory procedure to a fixed hidden platform. The groups did not differ in rate of learning ($F = 1.80$, $p > 0.10$). (**b**) All groups showed an equivalent tendency to swim in the training quadrant in the retention test 5 h later ($F = 1.97$, $p > 0.10$).

changes in glucose utilization predominantly reflect nerve terminal activity[28]. The remainder relates to ion fluxes and transport, which can be manipulated only after the inhibition of all electrical activity. In this study, the 23% decrease in glucose utilization in the dorsal hippocampus during LY infusion is similar to that observed after the suppression of synaptic transmission by pentobarbital[29,30]. Increasing the dose of LY might have further reduced the field-potential magnitude, 2-DG utilization or the volume of hippocampus affected, but at the risk of increasing extra-hippocampal diffusion. The primary site of action of our chronic LY infusions was the dorsal hippocampal formation, inclusive of areas CA1–CA3 and the dentate gyrus. The ventral (temporal) hippocampus was relatively unaffected, but this may be unimportant for the present learning task because similar spatial tasks are impaired more by dorsal than ventral neurotoxic lesions[31]. These electrophysiological and 2-DG data calibrate our claim that there would have been little or no residual cognitive function of the hippocampus during LY infusion but that normal function should return subsequently.

Encoding and retrieval

Turning to the dissociation between encoding and retrieval, we found that the only animals to perform above chance during recall were those given vehicle in both phases of training. This suggests that hippocampal activity is essential for both encoding- and retrieval-related memory processes—at least over a 16-day retention period. If LY had only impaired motor performance during training, masking normal memory encoding, the LY/vehicle animals should have performed well during the final retention test. LY treatment cannot, however, have only affected encoding-related processing because the vehicle/LY group was also impaired. The differential search patterns of these two groups are intriguing because, if hippocampal neurotransmission is involved in encoding, the vehicle/LY group would have been in a position to develop a localized search strategy during training, whereas the LY/vehicle group would not. However, the finding that the vehicle/LY group searched in a spatially localized way during the memory test, but in inappropriate locations, suggests that normal hippocampal activity is necessary to retrieve location information but is unnecessary to retrieve and execute the swimming strategy. Strategy information is most likely encoded and stored elsewhere. With respect to alternative explanations, the poor performance of the LY/LY treated group argues against state dependency. The localized searching of the vehicle/LY group argues against LY causing any motivational deficit during retrieval, and it therefore seems unlikely that motivation would be impaired during encoding either. An attentional account of chronic hippocampal inactivation remains possible, if implausible, and could be examined using tasks requiring vigilance or sustained attention[32].

We envisage that spatiotemporal patterns of glutamatergic or neuromodulatory activity in the hippocampus necessary to encode information into long-term memory must differ from those later required to retrieve it[33,34]. AMPA/kainate receptor blockade during encoding would necessarily prevent NMDA receptors being stimulated sufficiently to trigger the activity-dependent modifications of synaptic weights that constitute trace storage[11,12]. The integrity of transmission mediated by AMPA/kainate receptors in the hippocampus also seems to be necessary for retrieval. In contrast, NMDA receptor activation is necessary for encoding but not retrieval[35,36].

Consolidation or storage

We also found that memory traces are fragile to chronic reversible hippocampal inactivation starting one to five days after training and lasting seven days. Control experiments set various constraints on the interpretation of this finding. First, the impairment induced by chronic LY displays a measure of anatomical specificity. As predicted from rodent lesion studies (for example, ref. 37), hippocampal but not caudate inactivation causes retrogade amnesia for spatial memory. Second, inactivation for the limited period of 4–6 hours, beginning 24 hours after the end of training, did not impair memory. (If anything, it was paradoxically enhanced.) This is consistent with previous studies of acute post-training drug infusion in other tasks in which effects are generally seen at much shorter intervals[38]. Interference with short-term consolidation mechanisms is thereby excluded. Third, a residual deficit in a retrieval-related memory process once the hippocampus has switched back on was also excluded by the successful training of LY-treated animals in a second water maze task on the same day that they revealed impaired memory of the first task and by the null effects of reversible inactivation before all training in the main task. Thus, hippocampal neural activity is required for a memory-related process other than encoding or retrieval, which lasts for at least five days after the end of this training protocol.

At first sight, this finding suggests that hippocampal neural activity is necessary for the long-term consolidation of spatial memory traces. This interpretation is consistent with evidence for time-limited changes in glucose utilization in the hippocampus reflecting the energy demands of the putative consolidation process[39] and with lesion studies in humans and animals showing temporal gradients of retrograde amnesia[40–45]. However, an outstanding puzzle about this literature is that retrograde amnesia "...gradients do not provide a direct measure of the time required for the consolidation of long-term memory" (p 550, ref. 22). Specifically, two separate conceptions of long-term consolidation should be distinguished.

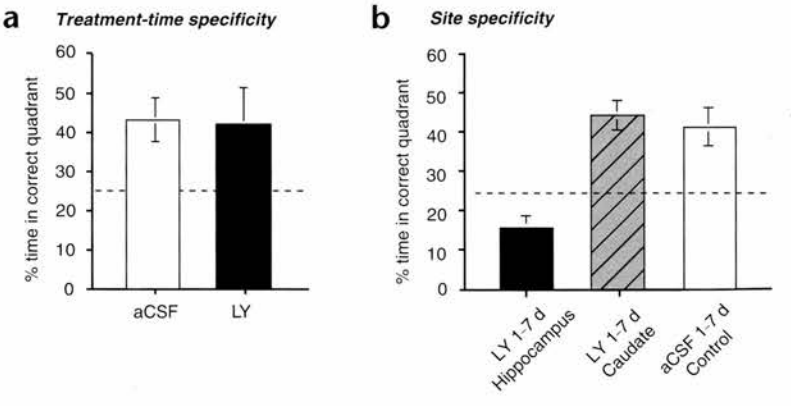

Fig. 6. The specificity of the effects of post-training hippocampal inactivation. (**a**) Treatment-time specificity was examined by implanting 7-day micropumps with either LY (n = 8) or vehicle (n = 8) and allowing them to infuse and become exhausted before the start of all training. Nine days later, the pretraining and spatial training protocol was conducted as in Fig. 4a. Both groups learned at an equivalent rate (F < 1; data not shown). Retrieval, tested 16 days later, showed equivalent levels of recall (F < 1). (**b**) Site specificity was examined by comparing LY infusions into the hippocampus (n = 8) and into the striatum (n = 8) one to seven days after spatial training. Only the intrahippocampal infusions impaired memory relative to the pooled vehicle controls (p < 0.01). Mean ± s.e.

One is that memory traces take time to be stabilized within one or more brain areas such that they can then persist for the lifetime of the animal; the other is that long-term consolidation is a network process through which traces stored in one brain area become resistant to brain damage elsewhere to which they are initially sensitive. These are distinct but not mutually exclusive ideas, excepting that the stabilization concept allows for the possibility that there is significant long-term storage of certain kinds of information in hippocampus (for example, context information) and that neural activity there always remains necessary for its retrieval[46].

Comparison of the effects of reversible inactivation and irreversible lesions provides one way of distinguishing these two accounts, specifically in a task such as the water maze in which a flat retrograde amnesia gradient is sometimes observed after permanent lesions. (See Table 2 in ref. 46.) Stabilization may still be occurring within hippocampus, even though hippocampal neural activity remains necessary both for retrieval and for aspects of performance during navigation, path-integration, etc. Unfortunately, our efforts to find the duration of stabilization/consolidation by extending the retention interval and delaying the start of inactivation until long after training have so far been unsuccessful because of poor memory baselines in vehicle-treated controls after retention intervals of up to 60 days. Other tasks may be more appropriate.

Without a temporal gradient, we are obliged to recognize the possibility that LY infusion, rather than affecting stabilization/consolidation, may disrupt the integrity of storage sites in the hippocampus for long-term spatial or contextual memory 'traces'[1,46]. Specifically, AMPA/kainate receptor blockade for seven days might disrupt spatially distributed patterns of synaptic weights, irrespective of how long after training it occurs. A possible mechanism would be the breakdown of the quantitative scaling of homeostatic plasticity. Quantitative scaling implies that the relative efficacy of different synapses is sustained in the face of partial AMPA receptor blockade and its cessation. Were this to occur *in vivo* also, the disruption to trace storage should be minimal. However, quantitative scaling may break down when sustained for several days, such that, when the micropump became exhausted, normal levels of field-potentials, neural excitability and glucose utilization would resume but the spatial pattern of altered synaptic weights might have been 'scrambled'. Immunogold labelling, ligand-binding and LTP experiments should be conducted to examine this idea rigorously.

Conclusion

In humans, functional imaging studies have taken us beyond classical lesion techniques to enable the identification of human brain structures differentially active during encoding and retrieval[47]. The hippocampus can be activated during encoding and retrieval[48,49], and these memory processes may occur preferentially at different points along its longitudinal axis[50]. In animals, reversible chronic inactivation of fast synaptic transmission complements neuron recording and other techniques by establishing, for each of several types of memory, whether the functional integrity of a brain area is necessary for a specific memory process.

Methods

***In vivo* electrophysiology.** This work was undertaken under the auspices of UK Home Office Project and Personal Licences held by the authors and designated laboratories. Male Lister hooded rats were anesthetized with urethane (acute experiments, 1.5 g per kg) or tribromoethanol (chronic experiments, 10 ml per kg). Using standard stereotaxic techniques, we implanted teflon-coated platinum-iridium electrodes (75 μm) to the appropriate depths for perforant path (AP –7.5 and L –4.0 mm; left hemisphere) or homotopic CA1 (AP –3.5 and L 2.0 mm) bipolar stimulation, and dentate (AP –3.5 and L –2.0 mm) or CA1 (AP –3.5 and L –2.0 mm) monopolar recording. Throughout surgery, all animals were placed on a heating blanket to maintain body temperature at 36.2 ± 0.2°C. In acute experiments, a stable baseline (20 min) was first obtained in response to electrical stimulation consisting of biphasic pulses with 100 μs half-width delivered at 0.05 Hz. Either vehicle or LY (1.0 μl of a 1.5 mM solution, concentration varied in pilot studies) was then infused through a stainless steel cannula (AP –4.5 and L –3.0 mm) attached by rubber tubing to a Hamilton syringe in a syringe driver. Recordings continued for another six hours. In chronic experiments, an infusion cannula was inserted into the hippocampus at AP – 4.5 and L –3.0 from which a catheter led to a minipump. Dental cement and stainless steel screws (one connected to the ground electrode) secured these to the skull. The minipump (ALZA2002) containing vehicle was placed in a cavity below the skin of the neck, and the length of tubing attached was calculated to contain fluid for 20 hours (at a pumping rate of 0.5 μl per hour). After a recovery period of 7–10 days, the awake animals were placed in a recording chamber where they could move about freely while electrically connected via a swivel commutator to signal-processing equipment. Daily recordings (3 days) included both input/output curves using stimulation varying from 100 to 1000 μA (100 μs half-width, 0.1 Hz) and 10-min baselines at a fixed stimulus intensity (50–70% of maximal response) with markers time stamped to positions on the trace. Under anesthesia, the minipump was then replaced with a micropump (ALZA1007, 0.5 μl per h for 7 days) containing either vehicle or LY (0.375 mM). Daily recordings continued in the same way for another 12 days.

Deoxyglucose measurements. Rats previously implanted (4 days or 11 days earlier) with intrahippocampal cannulae (AP –4.5 and L ±3.0 mm) and 7-day micropumps on both sides of the brain were anesthetized with halothane, and polyethylene cannulae were inserted into the right femoral vein and artery to allow the injection of [^{14}C]2-deoxyglucose and the sampling of blood, respectively. The cannulae were then passed subcutaneously and externalized at the nape of the neck. After at least 2 hours recovery, an intravenous pulse of 50 μCi [^{14}C]2-DG (specific activity 55.0 mCi per mol, Amersham Life Science) in 0.7 ml saline was injected over 30 s. Timed arterial blood samples (approximately 100 μl) were drawn at fixed time points over the next 45 minutes and concentrations of [^{14}C]2-DG and glucose in the blood samples determined. The rats were overdosed with euthatal 45 min later, and their brains removed and frozen. These were cut serially (3:10) into 20-μm thick coronal sections and autoradiograms generated by exposing the brain sections with medical X-ray film (Biomax™ MR film, Eastman Kodak Company), together with a series of precalibrated [^{14}C]-methyl methacrylate standards. Local rates of glucose utilization were determined with quantitative densitometric analysis using a computer-based densitometer (MCID, Imaging Research). Data from the 4- and 11-day groups were analyzed separately using 2-tailed Student's unpaired *t*-test.

Spatial learning. In the main task, rats were trained in an open-field water maze and tested for their memory of the escape platform location 16 days later. We used the 'Atlantis platform'[19], in which the polystyrene platform only became available from the bottom of the pool if the animal swam to and stayed within a 20 cm radius of the correct location for a predetermined and experimentally controllable dwell time. The platform then rose until its top surface is 1.5 cm below the water surface. This procedure encouraged highly focused searching. Pretraining (3 days, 6 trials per day; dwell time, 1 s on days 1 and 2, 1.5 s on day 3) consisted of approach to a visible hanging target above the location of the platform with the pool surrounded by curtains to occlude extramaze cues. Spatial training (4 days, 10 trials per day, dwell times of 1 s on days 1 and 2, 2 s on day 3 and 2.5 s on day 4) involved no hanging cue, and the curtains were drawn back to reveal extramaze cues. Swim paths and the times required to mount the hidden platform were monitored and stored on-line for later analysis. In the consolidation experiment, animals were assigned to groups matched with respect to their performance during the last training day. A single 60-s probe trial examined retention 16 days after the last training session. Surgery to implant acute infusion cannulae or micropumps was done at various times before or after the end of training as stated in the text. In the

encoding/retrieval experiment, all groups had their 14 day minipumps removed after training on day –1 (rather than day 0) so that any residual drug would be cleared within the next 24 h and would not interfere with any long-term consolidation process taking place after acquisition trials were completed.

All groups in the consolidation experiment were also trained in a second water maze located in a different laboratory on the retrieval test day (Day 16). There were 6 trials with a 30-s intertrial interval to a standard hidden platform in a fixed location, and a retention test (platform absent) 5 hours later. In the animals trained after the minipumps were exhausted, micropumps were inserted in both groups before all behavioral training in the main task. The group given bilateral caudate infusions was actually trained at the same time as the hippocampus-infused consolidation group.

Surgical procedures. Chronic bilateral implantation of cannulae and minipumps was done under general anesthesia (tribromoethanol) using standard stereotaxic techniques. Intrahippocampal cannulae (stainless steel, 26 gauge, L shaped) were connected via flexible polyethylene tubing to micropumps (ALZA 1007D). For hippocampal infusion, the cannulae tips were lowered to –3.0 mm below dura at coordinates of AP –4.5 and L ± 3.0 mm. For animals that received chronic and acute infusions at different times, the acute guide cannula (24 gauge) was soldered to the rostral end of the chronic one (26 gauge) with both tips level, so that the drug infused similar locations in the hippocampus. For caudate infusions, the coordinates were AP +0.7, L ±2.8, D –4.5 mm.

Histology. After termination of experimental procedures, brains of all animals were removed and histologically processed using a Nissl stain for verification of electrode and cannula placement. All data from a small number of animals with cannula misplacements or brain infections were discarded.

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